

Supporting Information for

**Selective chemical functionalization at N⁶-methyladenosine residues in DNA
enabled by visible-light-mediated photoredox catalysis.**

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Materials and Methods

General Remarks

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded at ambient temperature on a 400 MHz Bruker Avance III HD spectrometer (400 MHz) or a 500 MHz Bruker Avance III HD Smart Probe spectrometer (500 MHz). Chemical shifts (δ) were reported in ppm and quoted to the nearest 0.01 ppm relative to the residual protons in CDCl_3 (7.26 ppm), DMSO-d_6 (2.50 ppm), methanol- d_4 (3.31 ppm) and coupling constants (J) were quoted in Hertz (Hz). Coupling constants were quoted to the nearest 0.1 Hz and multiplicity reported according to the following convention: s = singlet, d = doublet, t = triplet, q = quartet, qnt = quintet, sxt = sextet, spt = septet, oct = octet, m = multiplet, br = broad and associated combinations, e.g. dd = doublet of doublets. Where coincident coupling constants have been observed, the apparent (app) multiplicity of the proton resonance has been reported. Data were reported as follows: chemical shift (multiplicity, coupling constants, number of protons and molecular assignment).

Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded at ambient temperature on a 400 MHz Bruker Avance III HD spectrometer (101 MHz) or a 500 MHz Bruker Avance III HD Smart Probe spectrometer (126 MHz). Chemical shifts (δ) were reported in ppm and quoted to the nearest 0.1 ppm relative to the residual solvent peaks in CDCl_3 (77.16 ppm), DMSO-d_6 (39.52 ppm), and methanol- d_4 (49.00 ppm). DEPT135, NOE experiments and 2-dimensional experiments (COSY, HMBC and HSQC) were used to support assignments when appropriate but were not included herein.

LCMS spectra were recorded on an Amazon X ESI-MS (Bruker) connected to an Ultimate 3000 LC (Dionex). Oligodeoxyribonucleotides were analysed using a gradient of 5-30% or 5-40% methanol vs. an aqueous solution of 10 mM triethylamine and 100 mM hexafluoro-2-propanol on a XTerra MS C18 column (125Å, 2.5 μm , 2.1x50mm) with TMS endcapping or an XBridge Oligonucleotide BEH C18 column (130Å, 2.5 μm , 2.1x50mm). Small molecules were analysed with a gradient of 0-100 % acetonitrile with 0.1% formic acid vs. water with 0.1% formic acid on a Kinetex® C18 column (100 Å, 2.6 μm , 50 x 2.1 mm). Mass chromatograms shown are base peak chromatograms, UV absorption was recorded at 260 nm.

High-resolution mass spectra (HRMS) of small molecules were conducted using Shimadzu LC-MS 9030 QToF. Oligodeoxyribonucleotides were analysed using a gradient of 5-30% methanol vs. an aqueous solution of 10 mM triethylamine and 100 mM hexafluoro-2-propanol on a XTerra MS C18 column (125Å, 2.5 μm , 2.1x50mm) with TMS endcapping. Small molecules were analysed with a gradient of 0-100 % acetonitrile with 0.1% formic acid vs. water with 0.1% formic acid on a Kinetex® C18 column (100 Å, 2.6 μm , 50 x 2.1 mm).

Analytical thin layer chromatography (TLC) was performed using pre-coated Merck glass-backed silica gel plates (Silicagel 60 F254 0.2 mm). Visualization was achieved using ultraviolet light (254 nm) and chemical staining with basic potassium permanganate solution as appropriate. Flash column chromatography was undertaken on Fluka or Material Harvest silica gel (230-400 mesh) under a positive pressure of air or on a CombiFlash Rf 200 (Teledyne Isco) system using 50 μm Si-HP PuriFlash columns.

Primer elongation reactions were performed on a T100 Thermocycler (BioRad) or on a 96 Universal Gradient (PeqStar) machine.

Automated gel electrophoresis was performed using an Agilent Technologies 2200 TapeStation and D1000 ScreenTapes and sample buffer.

qPCR was performed using a CFX96 Real-TimeSystem (BioRad), and data was processed using CFX software manager 3.1(BioRad). qPCR reactions (volume: 10 μ L) contained DNA calibration or sample mixtures (1 μ L), the corresponding forward and reverse primers (1 μ M each), and Brilliant III ultra-Fast SYBR green qPCR mastermix (Agilent Technologies, 5 μ L). Reactions were run according to the manufacturer's protocol. Calibration curves were made to determine the amounts of target DNA in the analysed samples.

Oligodeoxyribonucleotides (ODNs), including short ODNs for reactions, 99nt ssDNA strands and template and primers for the synthesis of 99nt dsDNA strands were custom synthesised and HPLC-purified by ATDBio or Sigma-Aldrich and used without further purification after dissolution into milliQ H₂O.

Reagents were obtained from Sigma-Aldrich, Acros, Alfa Aesar, TCI, or Jena Bioscience and used without further purification. Enzyme solutions were obtained from Zymo, New England BioLabs, and Sigma and used directly.

Dichloromethane, ethyl acetate, tetrahydrofuran, toluene, and petroleum ether (40–60) were dried and distilled using standard methods. Water was purified on a milliQ system. Other solvents used were purchased anhydrous and used without further purification unless otherwise stated.

Reactions were carried out under nitrogen atmosphere unless otherwise stated. Reactions were monitored by LCMS.

General procedures

General procedure A: functionalization of N⁶mdA in short ODNs with quinuclidine and derivatives

A 2 mL microwave vial was charged with a solution containing the ODN substrate (200 μ M in milliQ H₂O, 12.5 μ L). In a separate Eppendorf tube, the appropriate amount of quinuclidine or its derivative was dissolved using a stock solution of [Ru(bpz)₃](PF₆)₂ (2 mg in 1 mL of 20% MeCN in milliQ H₂O, 2.3 mM, 12.5 μ L) or [Ru(phen)₃]Cl₂ (2 mM in 20% MeCN in milliQ H₂O, 12.5 μ L). The latter mixture was then added to the ODN solution and the microwave vial was sealed under nitrogen atmosphere after flushing for 15-20 seconds. The vial was then placed at 5 cm from a 15 W CFL bulb. The reaction was irradiated for 5 hours, diluted with milliQ H₂O (25 μ L) and filtered through a prewashed Mini Quick Spin Oligo Column (Roche). The obtained mixture was analyzed directly by LCMS.

General procedure B: functionalization of N^6 mdA in short ODNs with 3-nitropyridine and derivatives

A 2 mL microwave vial was charged with a solution containing the ODN substrate (200 μ M in milliQ H₂O, 12.5 μ L). In a separate Eppendorf tube, quinuclidine (5.5 mg, 50 μ mmol) and the appropriate amount of 3-nitropyridine or respective derivatives were dissolved using a stock solution of [Ru(bpz)₃](PF₆)₂ (2 mg in 1 mL of 20% MeCN in milliQ H₂O, 2.31 mM, 12.5 μ L) or Ru(Phen)₃Cl₂ (1.5 mg in 1 mL of 20% MeCN in milliQ H₂O, 2.1 mM, 12.5 μ L). The latter mixture was then added to the oligo solution and the microwave vial was sealed under nitrogen atmosphere, flushing for 15-20 seconds. The vial was then placed in a distilled-water bath at approximately 1 cm from a 15 W CFL or 55 W CFL bulb or inside a 55W CFL bulb, in which case a fan was used for temperature control. The reaction was irradiated for 10 min (if not stated otherwise), diluted with water (25 μ L) and filtered through a prewashed Mini Quick Spin Oligo Column (Roche). The obtained mixture was analyzed directly by LCMS.

General procedure C: click reaction on the functionalized N^6 mdA in short ODNs

To the resulting ODN mixture from general procedure B (25 μ L) in a 1.5 ml Eppendorf tube was added quinuclidine (5.5 mg, 50 μ mol, dissolved in 10 μ L of milliQ H₂O), azide-PEG₃-biotin conjugate (20 mM, 5 μ L), sodium ascorbate (40 mM, 5 μ L) and CuSO₄ (2 mM, 5 μ L). Final concentrations: quinuclidine 1 M, azide-PEG₃-biotin conjugate: 2 mM, sodium ascorbate: 4 mM, CuSO₄ 0.2 mM. After 30 min reaction at room temperature, the reaction was filtered through a prewashed Mini Quick Spin Oligo Column (Roche). The obtained mixture was analyzed directly by LCMS.

General procedure D: enrichment of short N^6 mdA ODNs with streptavidin magnetic beads

To the resulting ODN mixture from general procedure C in a 1.5 ml Eppendorf DNA LoBind microcentrifuge tube was added 5X adapted binding buffer (40 μ L; 25 mM Tris, 2.5 mM EDTA, 5 M NaCl, pH 8.5, 0.25% Tween 20) and milliQ H₂O (for a final volume of 200 μ L). Streptavidin MagneSphere® Paramagnetic Particles (300 μ L of a 1 mg/ml suspension) were suspended in a 1.5 ml Eppendorf DNA LoBind microcentrifuge tube, separated from the storage buffer on a magnetic stand, washed twice with 0.5X SSC buffer (200 μ L; 75 mM NaCl, 7.5 mM sodium citrate, pH 7.2) and once with 1X adapted binding buffer (200 μ L; 5 mM Tris, 0.5 mM EDTA, 1 M NaCl, pH 8.5, 0.05% Tween 20). The oligo solution (200 μ L) was added to the paramagnetic particles and after 10 minutes incubation at r.t. the supernatant was collected for LCMS analysis. The beads were carefully washed with 1X adapted binding buffer (3x 200 μ L), then transferred into a new microcentrifuge tube with 200 μ L adapted binding buffer and washed another time with adapted binding buffer (1x 200 μ L). They were then incubated in 100 mM NaOH (3x 10 min at r.t. with 200 μ L), and finally washed again with adapted binding buffer (3x 200 μ L). Elution of the retained oligonucleotides was performed by incubation with 10% aqueous hydrazine (25 μ L) for 5 min at r.t. followed by an additional wash with water (25 μ L). Both fractions of 25 μ L were combined, filtered through a prewashed Mini Quick Spin Oligo Column (Roche) and analyzed directly by LCMS.

General procedure E: functionalization of N^6 mdA in 99nt ssDNA with the 3-nitropyridine probe for enrichment

A 2 mL microwave vial was charged with a mixture of two ODN substrates (methylated and unmethylated, 2 μ M in milliQ H₂O, 12.5 μ L each). In a separate Eppendorf tube, quinuclidine (8.25 mg, 75 μ mol) and the 3-nitropyridine probe (0.3 mg, 1.3 μ mol) were dissolved using a stock solution of [Ru(phen)₃]Cl₂ (3 mM in 1 mL of 30% MeCN in milliQ H₂O, 12.5 μ L). The latter mixture was then added to the ODN mixture. For the reactions with added salmon sperm DNA, 2 μ L of a salmon sperm ssDNA solution (5 mg/ml, abcam ab229278) were added before the photoredox reaction. Final concentrations: ODNs: 0.67 μ M each, quinuclidine: 2 M, 3-nitropyridine probe: 33 mM, [Ru(phen)₃]Cl₂: 1 mM, 10 % MeCN in H₂O. The microwave vial was sealed under nitrogen atmosphere after flushing for 15-20 seconds. The vial was then placed inside a 55W CFL bulb. The mixture was irradiated for 5 min while the temperature was maintained with a fan and subsequently filtered through a prewashed Micro BioSpin 6 column (BioRad) after the reaction.

General procedure F: click reaction on the functionalized N^6 mdA in 99nt ssDNA

To the resulting ODN mixture from general procedure E in a 1.5 ml Eppendorf tube was added quinuclidine (8.25 mg, 75 μ mol in 10 μ L H₂O), Azide-PEG₃-biotin conjugate (20 mM, 7.5 μ L), sodium ascorbate (40 mM, 7.5 μ L) and CuSO₄ (2 mM, 7.5 μ L). Final concentrations: quinuclidine: 1 M, Azide-PEG₃-biotin conjugate: 2 mM, sodium ascorbate: 4 mM, CuSO₄: 0.2 mM. After 30 minutes reaction at r.t., the mixture was filtered twice through a prewashed Micro BioSpin 6 column (BioRad).

General procedure G: functionalization of N^6 mdA in 99bp dsDNA with the 3-nitropyridine probe for enrichment

A 2 mL microwave vial was charged with a mixture of two dsODN substrates (methylated and unmethylated, 0.25-0.35 μ M in milliQ H₂O, 8.3 μ L each). In a separate Eppendorf tube, quinuclidine (5.5 mg, 50 μ mol) and the 3-nitropyridine probe (0.2 mg) were dissolved using a stock solution of [Ru(phen)₃]Cl₂ (3 mM in 1 mL of 30% MeCN in milliQ H₂O, 8.3 μ L). The latter mixture was then added to the ODN mixture. For the reactions with added salmon sperm DNA, 2 μ L of a salmon sperm ssDNA solution (5 mg/ml, abcam ab229278) were added before the photoredox reaction. Final concentrations: ODNs: 0.08-0.12 μ M each, quinuclidine: 2 M, 3-nitropyridine probe: 33 mM, [Ru(phen)₃]Cl₂: 1 mM, 10 % MeCN in H₂O. The microwave vial was sealed under nitrogen atmosphere after flushing for 15-20 seconds. The vial was then placed inside a 55W CFL bulb. The mixture was irradiated for 5 min while the temperature was maintained with a fan and subsequently filtered through a prewashed Micro BioSpin 6 column (BioRad) after the reaction.

General procedure H: click reaction on the functionalized N^6 mdA in 99bp dsDNA

To the resulting ODN mixture from general procedure G in a 1.5 ml Eppendorf tube was added quinuclidine (5.5 mg, 50 μ mol in 5 μ L H₂O), Azide-PEG₃-biotin conjugate (20 mM, 7.5 μ L),

sodium ascorbate (40 mM, 7.5 μ L) and CuSO₄ (2 mM, 7.5 μ L). Final concentrations: quinuclidine: 1 M, Azide-PEG₃-biotin conjugate: 2 mM, sodium ascorbate: 4 mM, CuSO₄: 0.2 mM. After 30 minutes incubation at r.t., the reaction was filtered twice through a prewashed Micro BioSpin 6 column (BioRad).

General procedure I: enrichment of N⁶mdA 99nt ssDNA and dsDNA ODNs with streptavidin magnetic beads

To the resulting ODN mixture from general procedures F and H (75 or 50 μ L) in a 1.5 ml Eppendorf DNA LoBind microcentrifuge tube was added 5X adapted binding buffer (40 μ L; 25 mM Tris, 2.5 mM EDTA, 5 M NaCl, pH 8.5, 0.25% Tween 20), poly[dI:dC] (5 μ L; 5 μ g/ μ l), and water (80 or 105 μ L, to a total volume of 200 μ L). Streptavidin MagneSphere® Paramagnetic Particles (100 μ L of a 1 mg/ml suspension) were separated from the storage buffer on a magnetic stand, washed twice with 0.5X SSC buffer (200 μ L; 75 mM NaCl, 7.5 mM sodium citrate, pH 7.2) and once with 1X adapted binding buffer (200 μ L; 5 mM Tris, 0.5 mM EDTA, 1 M NaCl, pH 8.5, 0.05% Tween 20) with poly[dI:dC] (5 μ L; 5 μ g/ μ l). The oligo solution (200 μ L) was added to the prewashed paramagnetic particles and after 10 minutes incubation at r.t. the supernatant was collected for the quantification of both ODN sequences by qPCR. The beads were washed three times with 1X adapted binding buffer (3x 200 μ L), then transferred into a new microcentrifuge tube with 200 μ L adapted binding buffer and washed another time with adapted binding buffer (200 μ L). They were then incubated in 100 mM NaOH at r.t. for 10 min three times (3x 200 μ L), and finally washed again three times with adapted binding buffer (3x 200 μ L). Elution of the bound oligonucleotides was performed by incubation with 10% aqueous hydrazine (25 μ L) at r.t. for 5 min. After an additional wash of the paramagnetic particles with water (25 μ L), both fractions were combined, purified using Zymo Oligo Clean & Concentrator™ spin columns (according to the manufacturers protocol) and the oligonucleotides eluted with 15 μ L milliQ water. 7.5 μ L of this purified fraction was kept for the determination of enrichment factors by quantifying both ODN sequences by qPCR. The other 7.5 μ L were diluted with 5X adapted binding buffer (10 μ L) and water (32.5 μ L) and treated with prewashed Streptavidin MagneSphere® Paramagnetic Particles (100 μ L of a 1 mg/ml suspension) to remove any residue of unselectively biotinylated oligonucleotides. The supernatant was purified using Zymo Oligo Clean & Concentrator™ spin columns according to the manufacturers protocol and the oligonucleotides eluted with 15 μ L milliQ water. The enrichment factor was determined by quantifying both ODN sequences by qPCR.

Supplementary Text and Figures

Important remarks on the photocatalyst used

While the design plan in Figure 2 of the main manuscript describes the use of $\text{Ru}(\text{phen})_3\text{Cl}_2$ photocatalyst and its oxidative quenching cycle, many of the preliminary results of this project were conducted using similar photocatalyst, $[\text{Ru}(\text{bpz})_3](\text{PF}_6)_2$. Due to the difference in the reduction potentials, the operative photoredox cycle for the latter is more likely to be the reductive quenching cycle: oxidation of the quinuclidine **3** as first step and reduction of 3-nitropyridine **1a** to restore the metal complex ground state (Fig. S1). It is important to note that while the photoredox cycles are different, the radical species formed are the same (**int I** and **int III**); indeed, both the photocatalysts showed very similar yields and reaction profile on short DNA fragments. However, the difference in the quenching cycle was crucial for the application of the reaction to single stranded (ss) and double stranded (ds) long oligonucleotides, as the $\text{Ru}(\text{phen})_3\text{Cl}_2$ showed significantly cleaner reaction profiles due to lower oxidative power compare to $[\text{Ru}(\text{bpz})_3](\text{PF}_6)_2$ (Fig. S16).

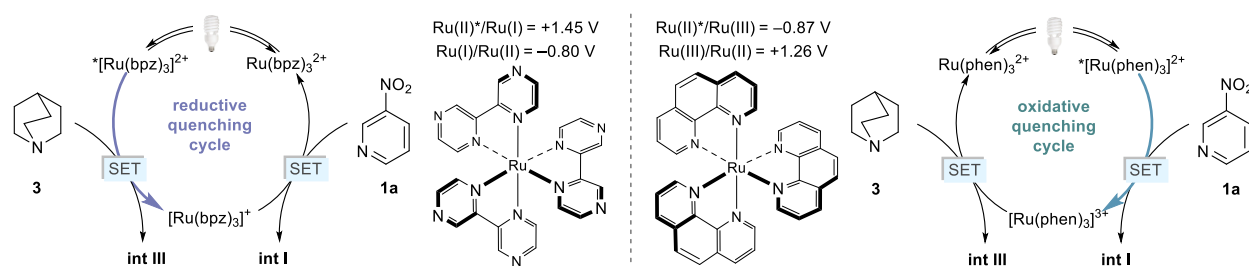


Fig. S1. Proposed photoredox quenching cycles for $[\text{Ru}(\text{bpz})_3](\text{PF}_6)_2$ and $\text{Ru}(\text{phen})_3\text{Cl}_2$.

Reactions with quinuclidine and quinuclidine derivatives

Reaction with quinuclidine on methylated/unmethylated ODNs

Initial studies demonstrated that irradiation of a mixture of ODN **5** with quinuclidine **3** and the photosensitiser [Ru(bpz)₃](PF₆)₂ resulted in the formation of two products as detected by LCMS (Fig. S2). Whereas retention time and MS profile of the first product clearly indicated that it is the demethylation product [dA] **7**, the other product had the mass of a putative quinuclidine derivative [N⁶mdA-Q].

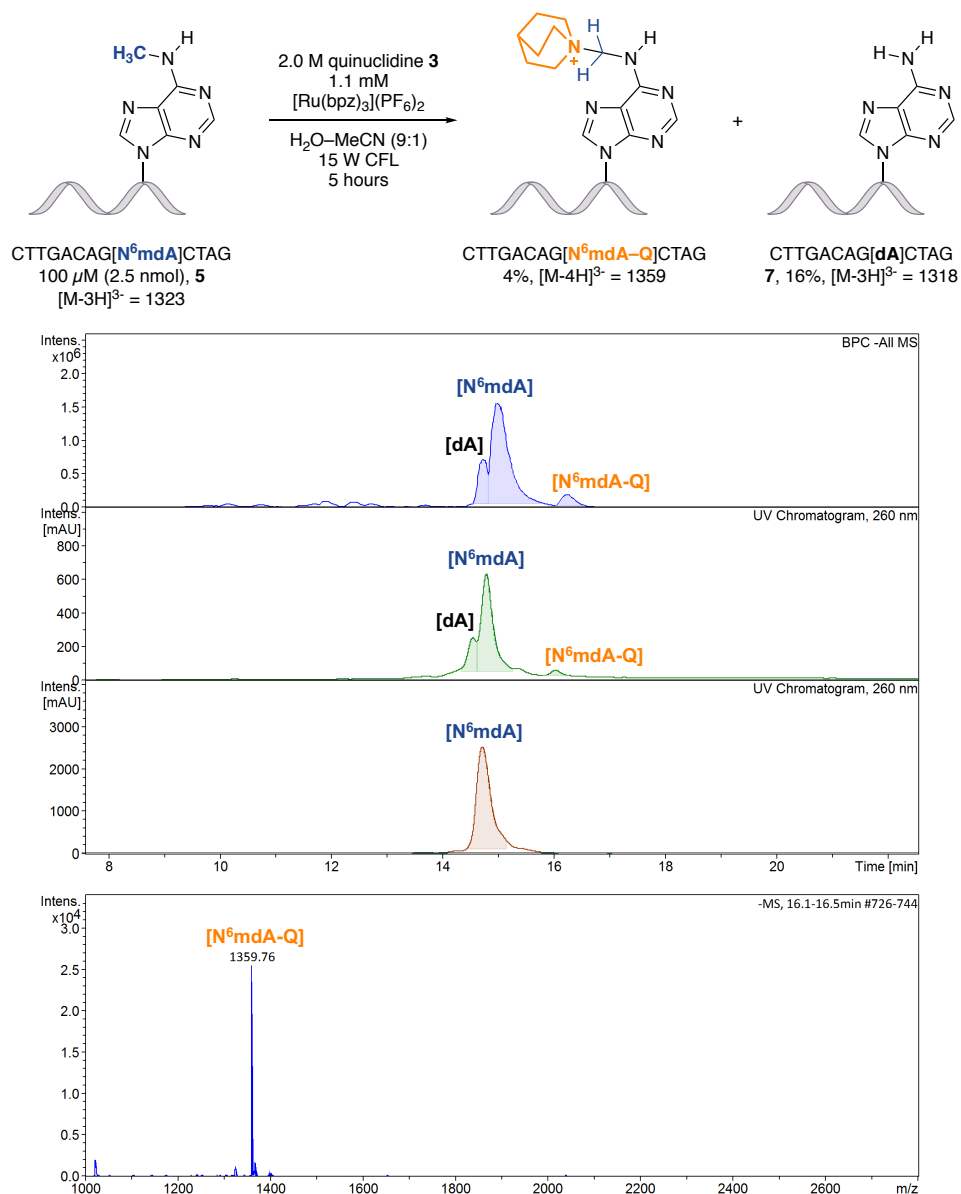


Fig. S2. Reaction scheme for the selective N⁶mdA functionalization reaction on the methylated ODN **5**, LCMS traces of the reaction mixture after the reaction (top: base peak mass chromatogram, middle: UV₂₆₀ chromatogram) and of the starting material (UV₂₆₀ chromatogram; bottom), and extracted mass spectrum of the [N⁶mdA-Q] peak.

When substituting substrate ODN **5** by its demethylated analogue **7** and applying the same conditions, no reaction could be observed (Fig. S3).

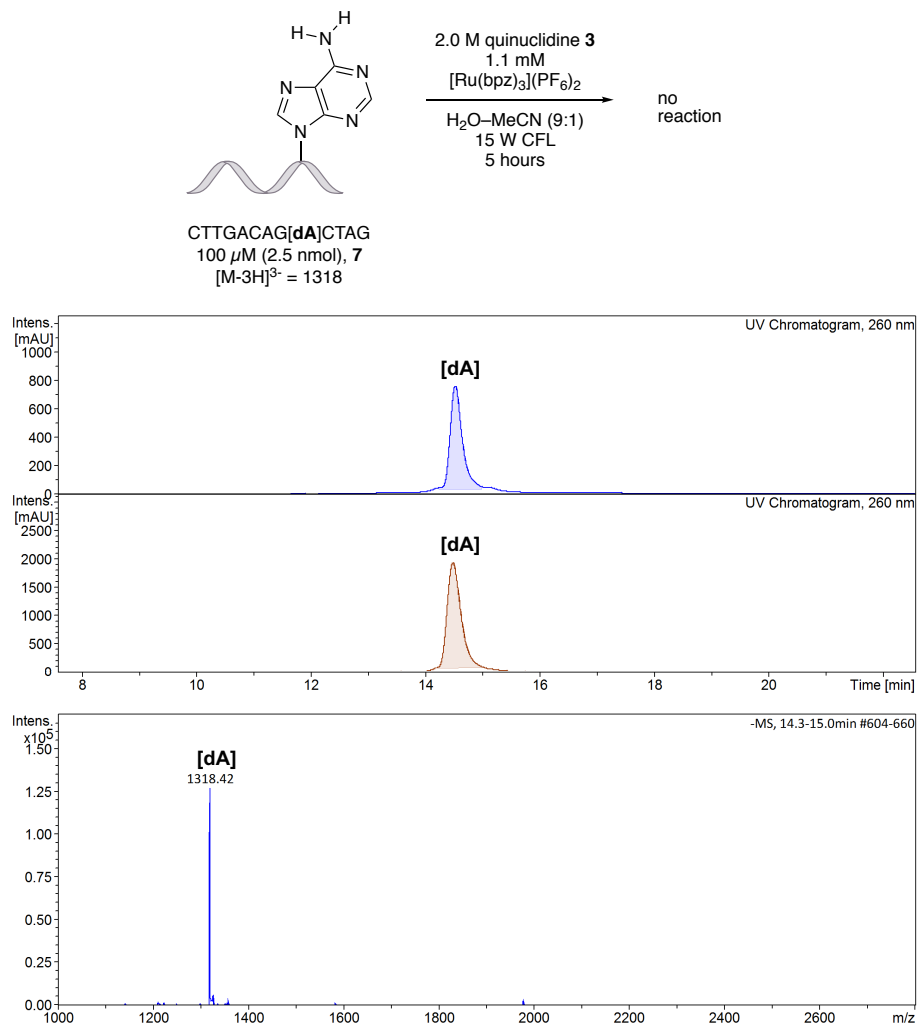


Fig. S3. Reaction scheme for the application of the N⁶mdA-selective functionalization conditions using quinuclidine **3** on the unmethylated ODN **7**, LCMS traces of the reaction mixture after the reaction (UV₂₆₀ chromatogram; top) and of the starting material (UV₂₆₀ chromatogram; bottom), and extracted mass spectrum of the [dA] peak (ODN **7**) after reaction.

This set of experiments led to the conclusion that both observed products in the reaction on ODN **5** are the result of a selective reaction at the N⁶mdA residue. We posit that both are the product of an initial highly selective HAT from the N-methyl group to the well-described quinuclidinium radical cation formed *in situ*.

Reaction with quinuclidine derivative 2-(quinuclidin-3-yl)propan-2-ol

Further confirmation of the incorporation of quinuclidine on the N⁶mdA residue was shown by developing a quinuclidine derivative that is compatible with the reaction in Fig. S2: 2-(Quinuclidin-3-yl)propan-2-ol. Under similar conditions to the quinuclidine functionalization in Fig. S2, we observed the new product [N⁶mdA-Qder] along with product 7 (Fig. S4).

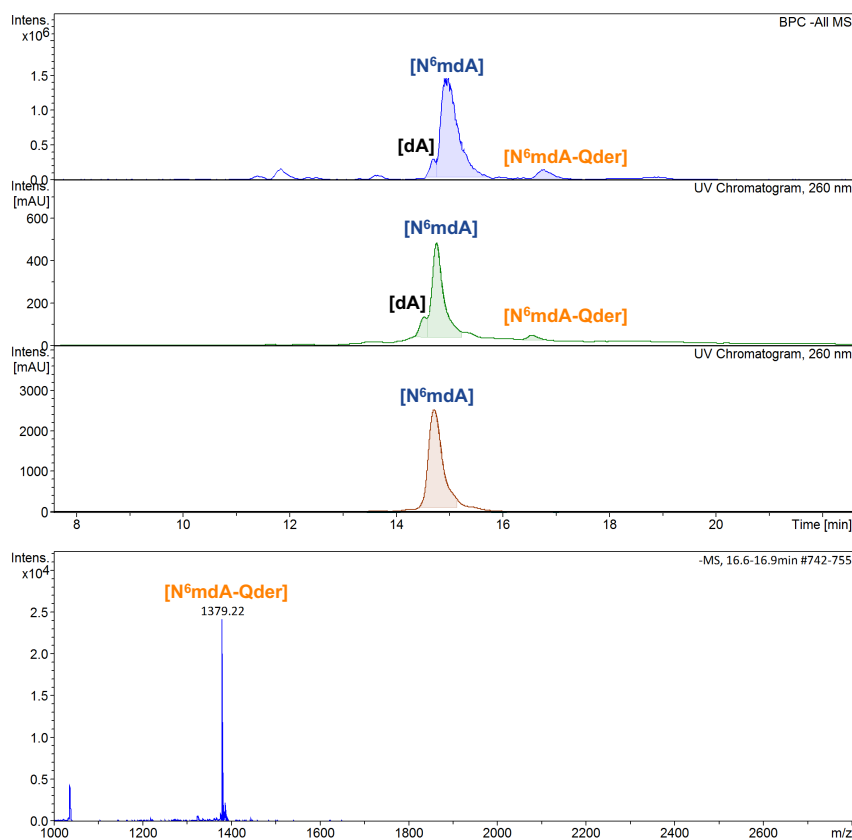
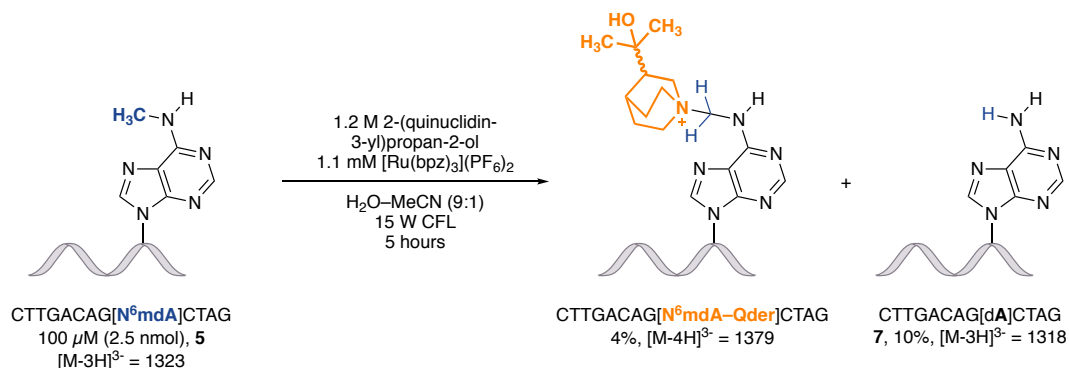


Fig. S4. Reaction scheme for the selective N⁶mdA functionalization reaction with a developed quinuclidine derivative on methylated ODN 5; LCMS traces of the reaction mixture after the reaction (top: base peak mass chromatogram, middle: UV₂₆₀ chromatogram) and of the starting material (UV₂₆₀ chromatogram; bottom), and extracted mass spectrum of the [N⁶mdA-Qder] peak.

This experiment confirms that the second observed product (as shown in Fig. S4) is a quinuclidine derivative.

Reactions with [Ru(bpz)₃](PF₆)₂ and reduced concentrations of quinuclidine

To understand if excited [Ru(bpz)₃](PF₆)₂ can damage ODNs in the absence of high amounts of quinuclidine, we run control reaction on ODN **5** (as depicted in the scheme of Fig. S2) with decreasing concentrations of quinuclidine **3** (Fig. S5).

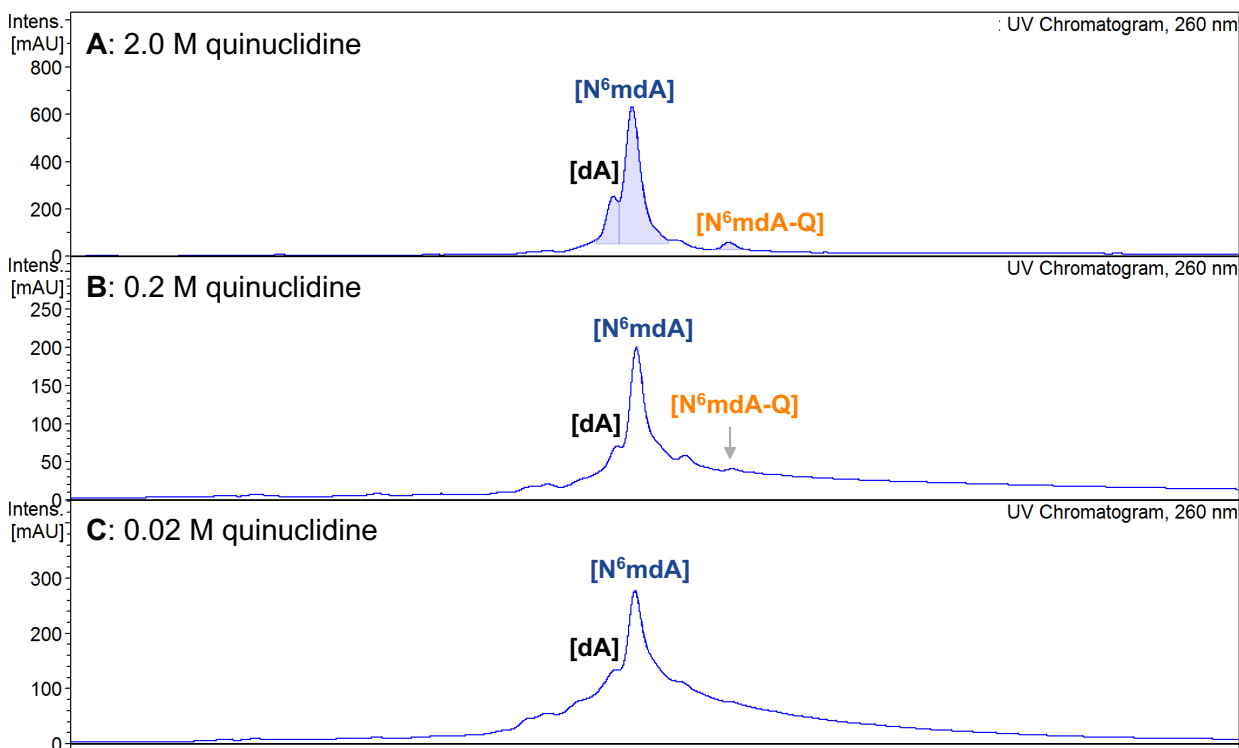


Fig. S5. LCMS UV₂₆₀ traces of the reaction mixture after reaction (according to General Procedure A) with different concentrations of quinuclidine **1**. **A:** 2.0 M quinuclidine. **B:** 0.2 M quinuclidine. **C:** 0.02 M quinuclidine. Considerable broadening of the LCMS peaks in the lower chromatograms indicates decomposition of the ODNs at reduced concentrations of quinuclidine.

Considerable broadening of the LCMS peaks when using lower quinuclidine concentrations indicates that decreasing quinuclidine concentration is detrimental to the selectivity of the reaction.

LCMS studies on the formation of the N-hydroxyformamidinium product

Encouraged by the validation of the HAT step, we next investigated the *in situ* generation of the nitrosoarene spin trapping reagent **2a** from 3-nitropyridine **1a** and its interception of the N⁶mdA-derived α -amino radical. We found that irradiation of a solution of oligonucleotide **5**, quinuclidine **3**, [Ru(bpz)₃](PF₆)₂ and 3-nitropyridine **1a** for 10 minutes at room temperature produced N-hydroxyformamidinium conjugate [N⁶mdA-P] **6** in 14% yield (Fig. S6), along with quinuclidine product [N⁶mdA-Q] (3% yield) and demethylation product [dA] **7** (26% yield).

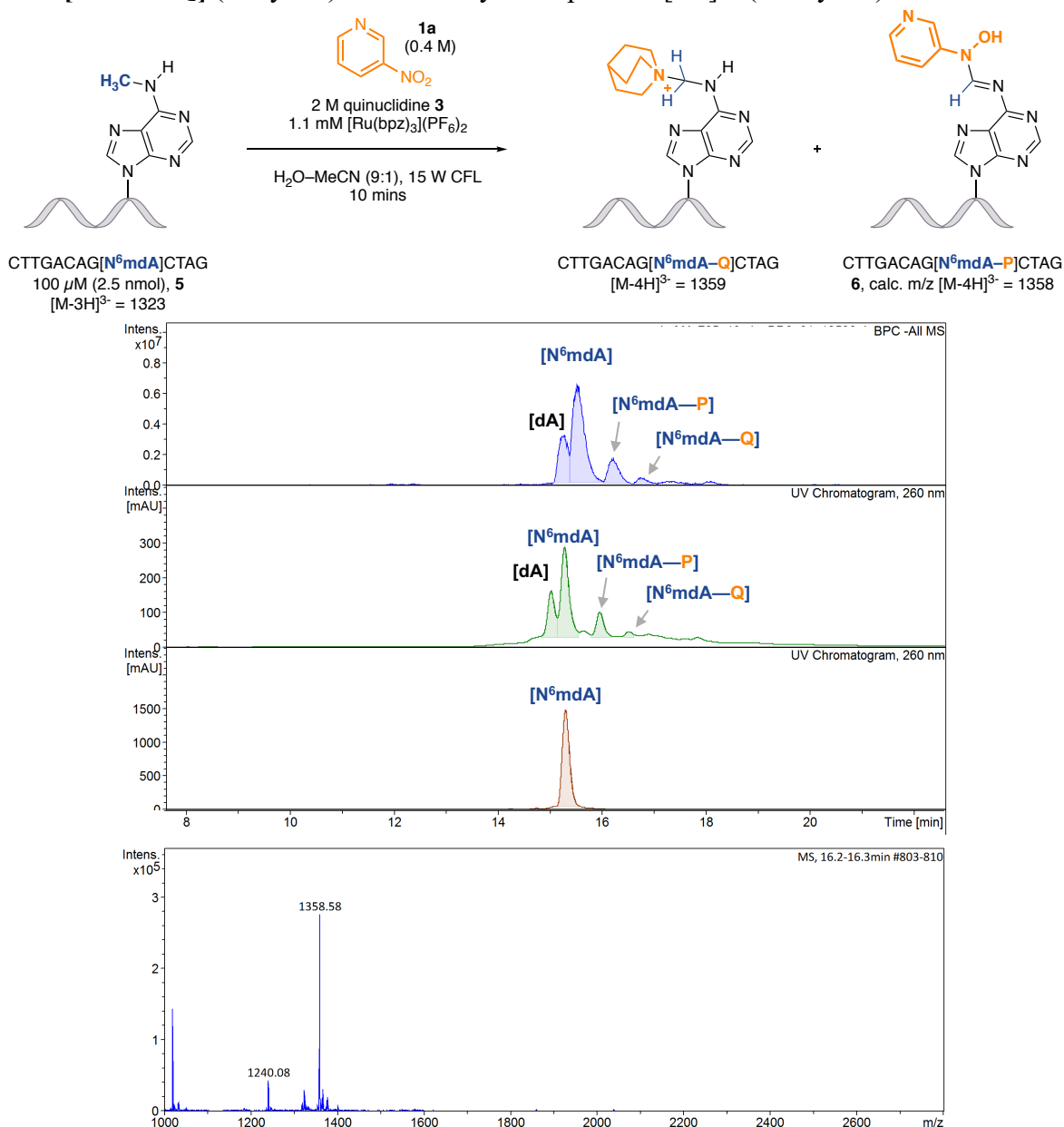


Fig. S6. Reaction scheme for the selective N⁶mdA functionalization reaction on methylated ODN **5** in the presence of 3-nitropyridine (**1a**); detection of the new N-hydroxyformamidinium product [N⁶mdA-P] **6** (in 14% assay yield) with m/z 1358. LCMS traces show the reaction mixture after the reaction (top: base peak mass chromatogram, middle: UV₂₆₀ chromatogram) and the starting material (UV₂₆₀ chromatogram; bottom). The MS shows the extracted mass spectrum of the [N⁶mdA-P] **6** peak.

Given the fact that the observed mass of N-hydroxyformamidinium conjugate **[N⁶mdA-P] 6** ($m/z = 1358$) is very similar to the mass of quinuclidine product **[N⁶mdA-Q]** ($m/z = 1359$), we conducted a control experiment using 5-Phenyl-3-nitropyridine (Fig. S7) to confirm that the new product **6** is indeed a derivative of 3-nitropyridine **1a** and not the oxidized version of quinuclidine product **6**. When 5-phenyl-3-nitropyridine was used under similar reaction conditions showed in Fig. S5, we observed the formation of a new product **[N⁶mdA-P-Ph]** with $m/z = 1384$ in 5% yield, quinuclidine product **[N⁶mdA-Q]** (2% yield) and demethylated adduct **[dA] 7** (15% yield). The mass of the new product **[N⁶mdA-P-Ph]** clearly reflects the incorporation of 5-Phenyl-3-nitropyridine and, together with the fact that no peak with $m/z = 1358$ was detected, it unambiguously confirms that product **[N⁶mdA-P] 6** is indeed a 3-nitropyridine **1a** derivative.

product with m/z 1358 **not** present

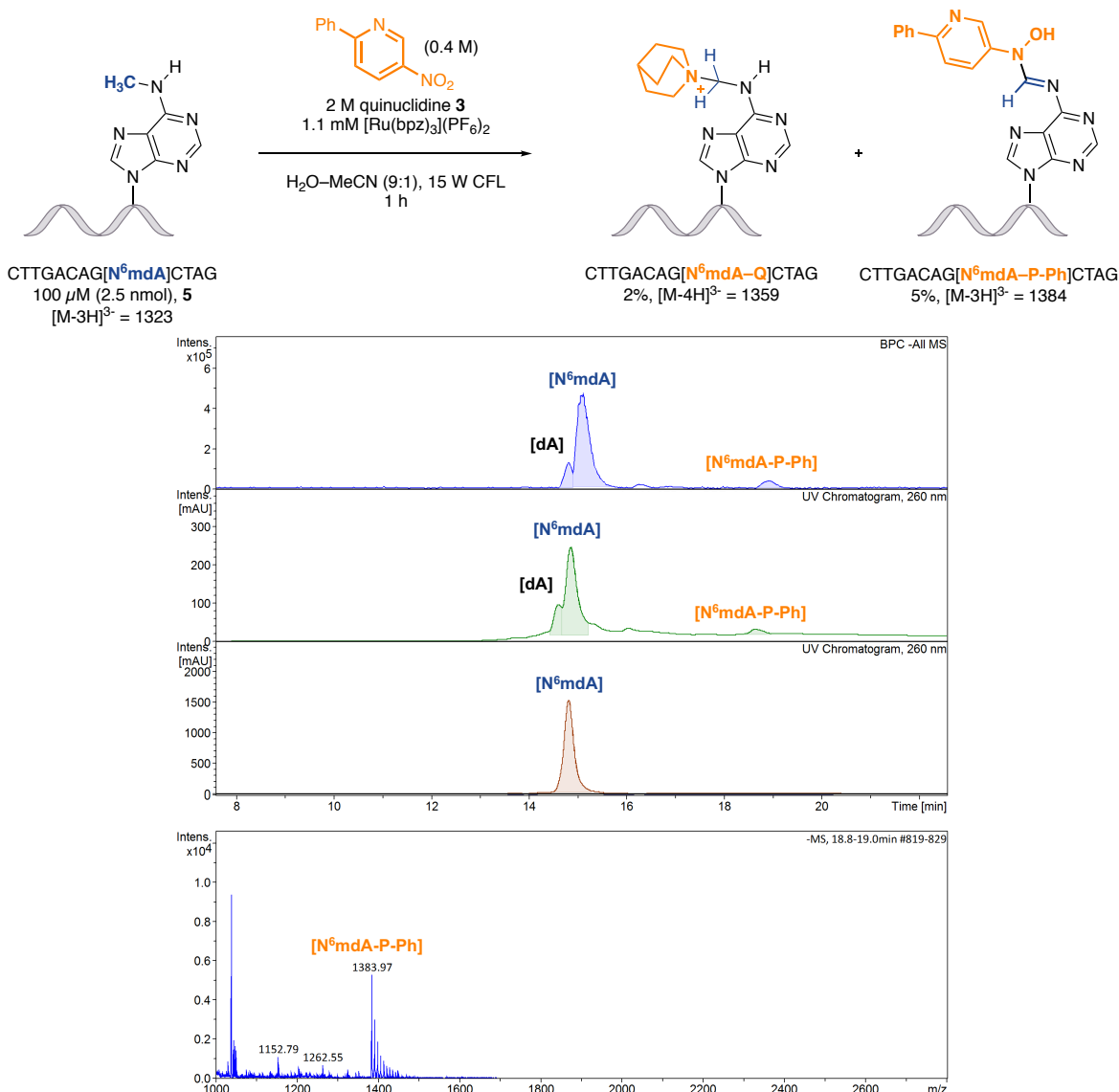


Fig. S7. N⁶mdA-selective functionalization of N⁶mdA ODN **5** with quinuclidine **1** and 2-phenyl-5-nitropyridine resulted in a product with m/z 1384 that corresponds to incorporation of a 2-phenyl-5-nitrosopyridine moiety **[N⁶mdA-P-Ph]** along with the previously described quinuclidine product **[N⁶mdA-Q]**. LCMS traces show the reaction mixture after the reaction (top: base peak mass chromatogram, middle: UV₂₆₀ chromatogram) and the starting material (UV₂₆₀ chromatogram; bottom). The MS shows the extracted mass spectrum of the **[N⁶mdA-P-Ph]** product peak.

Additional evidences are provided by the following experiment, where we used 3-nitropyridine **1a** and 2-(Quinuclidin-3-yl)propan-2-ol instead of quinuclidine **3** (Fig. S8). In this case, we could detect the formation of product $[N^6\text{mdA-P}]$ **6** with $m/z = 1358$ (5% yield) and the 2-(Quinuclidin-3-yl)propan-2-ol product $[N^6\text{mdA-Qder}]$, but we could not observe the formation of the previously observed quinuclidine product $[N^6\text{mdA-Q}]$ as expected. This experiment clearly confirms that product $[N^6\text{mdA-P}]$ **6** is not the mere oxidized version of quinuclidine product.

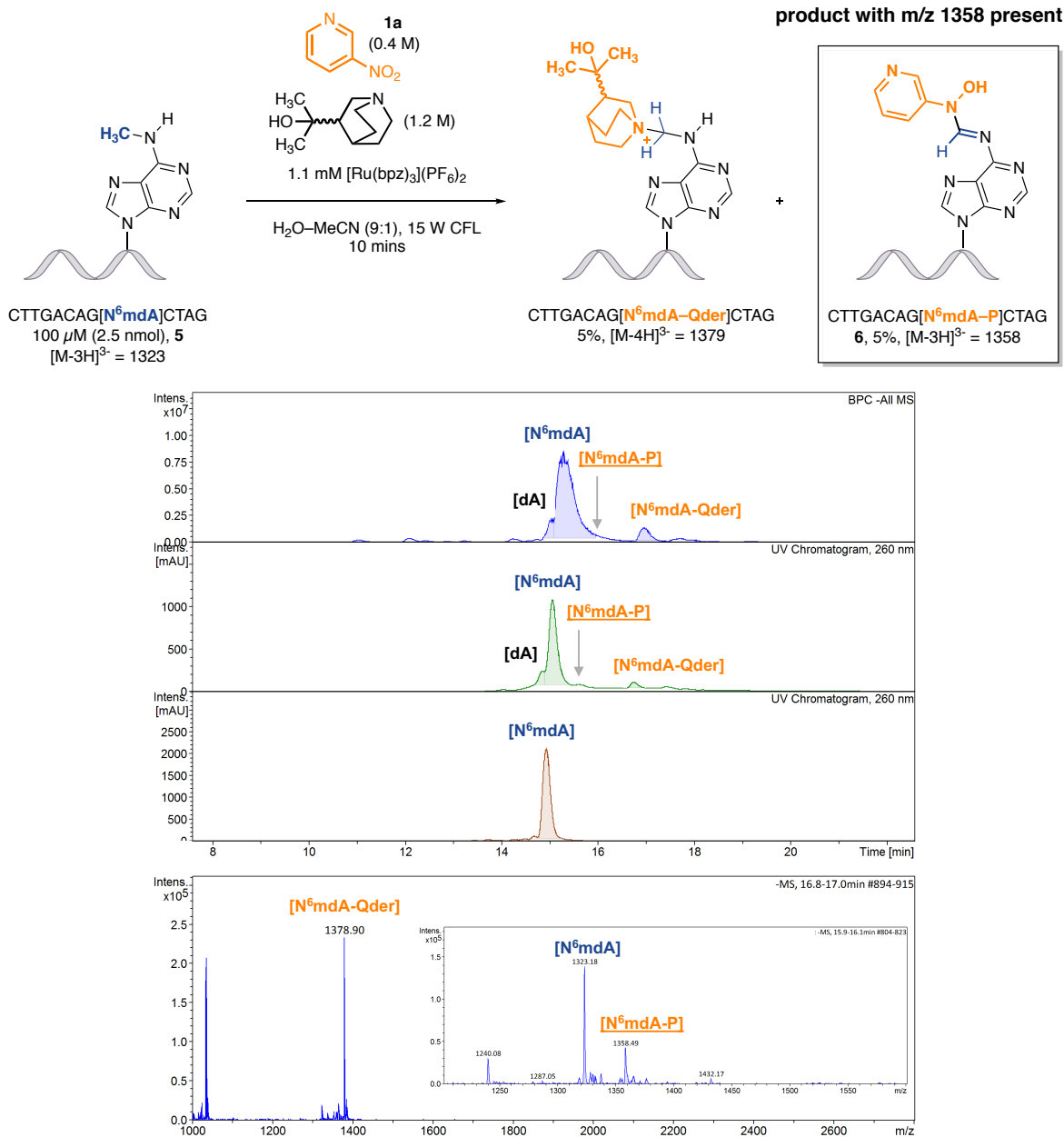


Fig. S8. $N^6\text{mdA}$ -selective functionalization of $N^6\text{mdA}$ ODN **5** with the quinuclidine derivative 2-(quinuclidin-3-yl)propan-2-ol and 3-nitropyridine (**2a**) resulted in the product with m/z 1358 that corresponds to incorporation of a 3-nitrosopyridine moiety $[N^6\text{mdA-P}]$ **6** along with the product with the derivatized quinuclidine $[N^6\text{mdA-Qder}]$. LCMS traces show the reaction mixture after the reaction (top: base peak mass chromatogram, middle: UV₂₆₀ chromatogram) and the starting material (UV₂₆₀ chromatogram; bottom). The MS shows the extracted mass spectrum of the $[N^6\text{mdA-Qder}]$ product peak as well as of the $[N^6\text{mdA-P}]$ **6** product region (insert).

Selective functionalization of N⁶mdA ODN **5** with derived 3-nitropyridines

Various 3-nitropyridine derivatives have been tested under the N⁶mdA selective reaction conditions described in Fig. S6. The aim of this study was to provide information regarding the solubility, reactivity and compatibility of modified 3-nitropyridine compounds toward the development of a probe that can contain an alkyne group for future enrichment studies.

Table S1. Conditions and yields for the selective functionalization reactions of N⁶mdA ODN **5** with different 3-nitropyridine derivatives.

N⁶mdA functionalization
 2 M quinuclidine **3**
 3-nitropyridine der.
 1.1 M [Ru(bpz)₃](PF₆)₂
 H₂O–MeCN (9:1), 15 W CFL
 5-60 mins

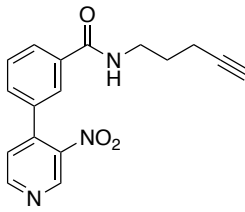
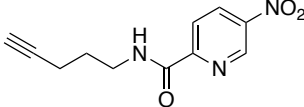
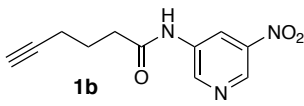
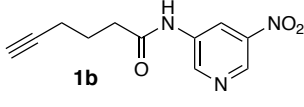
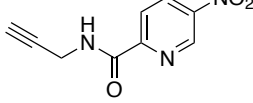
+ CTTGACAG[dA]CTAG
7, [M-3H]³⁺ = 1318
 + CTTGACAG[N⁶mdA-Q]CTAG
 [M-4H]³⁺ = 1359

CTTGACAG[N⁶mdA]CTAG
5, (100 uM)
 [M-3H]³⁺ = 1323

CTTGACAG[N⁶mdA-PX]CTAG

3-nitropyridine der.	[c]	reaction time ^a	yield [N ⁶ mdA-PX] ^b (m/z [M-3H] ³⁺)	yield [dA] ^b	yield [N ⁶ mdA-Q] ^b
	0.4 M	1 h 15 W CFL	5 % (1384)	15 %	2 %
	0.4 M	1 h 15 W CFL	7 % (1384)	17 %	2 %
	0.4 M	10 min 15 W CFL	4 % (1385)	38 %	3 %
	0.2 M	1 h 15 W CFL	2 % (1420)	11 %	3 %
	0.13 M	1 h 15 W CFL	1 % (1420)	19 %	2 %
	0.13 M	1 h 15 W CFL	2 % (1420)	13 %	2 %

Table S1 continued

derived 3-nitropyridine	[c]	reaction time ^a	yield [N ⁶ mdA-PX] ^b (m/z [M-3H] ³⁺)	yield [dA] ^b	yield [N ⁶ mdA-Q] ^b
	0.08 M	1 h 15 W CFL	3 % (1420)	28 %	2 %
	0.1 M	20 min 15 W CFL	7 % (1394)	35 %	1 %
	0.1 M	20 min 15 W CFL	8 % (1394)	17 %	5 %
	0.1 M	20 min 60 W CFL	17 % (1394)	37 %	2 %
	0.1 M	20 min 15 W CFL	8 % ^c (1385)	25 %	2 % ^c

^a other reaction conditions according to General Procedure B

^b assay yields by LCMS

^c species coeluting in LCMS, approximate yields according to MS signal intensities

We first started our exploration using 3-nitropyridine derivatives containing a phenyl group as substituent or linker for the alkyne moiety. The choice of the phenyl group was done based on the small changes in the reduction potential compare to unsubstituted 3-nitropyridine. Indeed, 3-nitropyridine needs to be reduced to generate the desired spin trapping reagent 3-nitrosopyridine; therefore, a phenyl substituent would not compromise the key reduction step. Unfortunately, all the substrates containing an aromatic substituent were poorly soluble in the aqueous media and showed low yields for the selective functionalization of N⁶mdA. Later, we found that the amide linkage was well tolerated in position 4 and 5 of the 3-nitropyridine, furnishing the desired functionalisation in better yields. Finally, we could improve the initial result of 3-nitropyridine **1b** (8% yield) using a more powerful source of light, such as 60W CFL (17% yield).

LCMS traces of functionalization of N⁶mdA ODN 5 and control reaction with unmethylated ODN 7 using different alkyne-derived 3-nitropyridines

This is a collection of LC traces of the selective photoredox reactions on N⁶mdA containing ODN 5 described in **Table S1** using different 3-nitropyridine alkyne derivatives (probes) and the corresponding control experiments on unmethylated ODN 7 under the same reaction conditions. For each probe, the first chromatogram is the MS-trace of the reaction on [N⁶mdA] ODN 5. The second chromatogram shows the UV-trace of the same reaction. The third and the fourth chromatograms represent respectively the MS and UV-traces of the control experiment on unmethylated [dA] ODN 7. Finally, the last trace is the MS spectra of the new product peak observed in the first two traces.

While the formation of a new product is evident for all the probes tested on [N⁶mdA] ODN 5, control experiments showed clean recovery of unmethylated [dA] ODN 7 in all cases, without the presence of any N-hydroxyformamidinium adducts. **These results not only indicate an exquisite selectivity for the functionalisation of N⁶mdA, but also unambiguously confirm the position of the new label on the N⁶ methyl group.**

N-(3-(5-nitropyridin-2-yl)phenyl)hex-5-ynamide

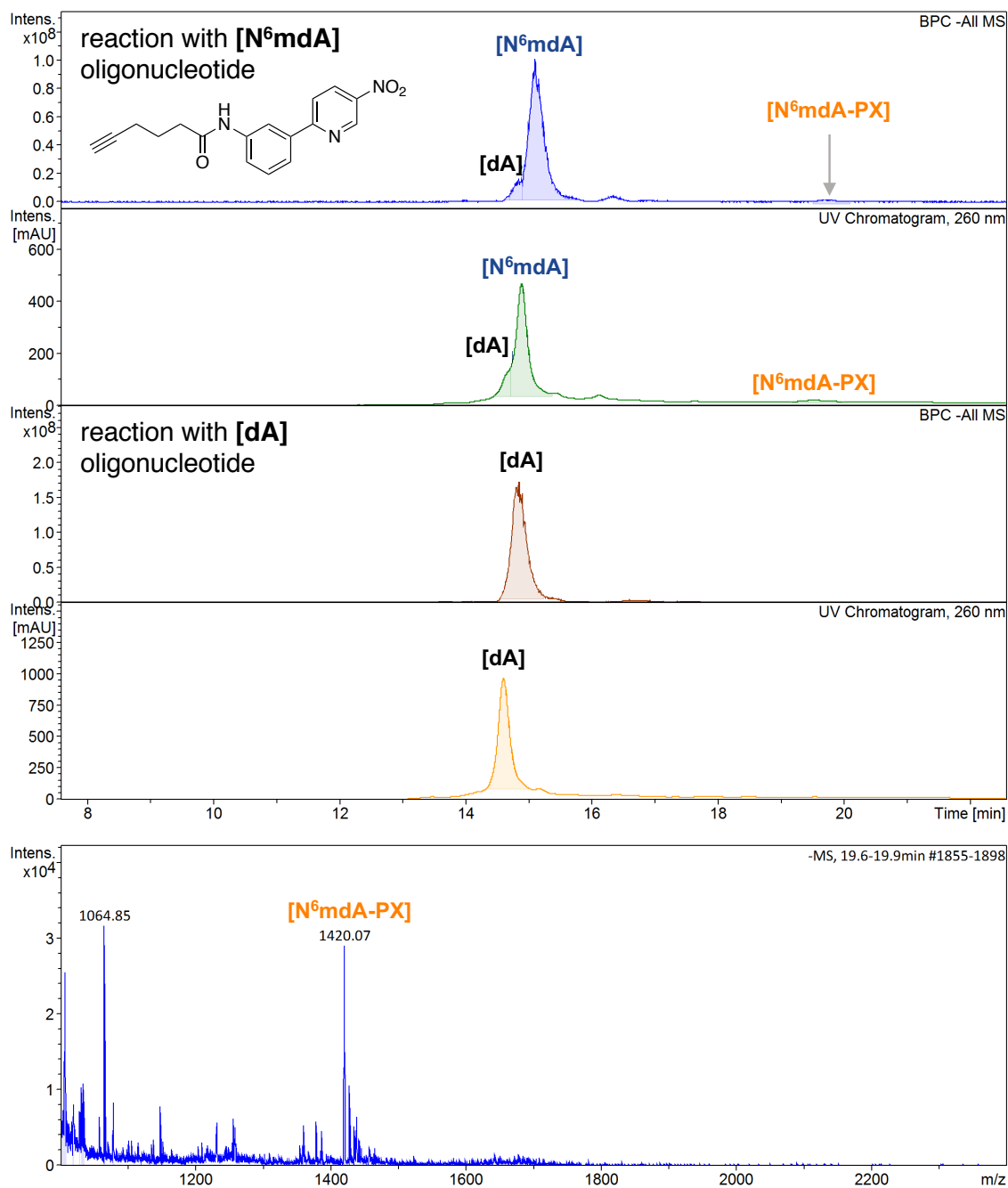


Fig. S9. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with *N*-(3-(5-nitropyridin-2-yl)phenyl)hex-5-ynamide on N⁶mdA ODN 5 (top; base peak MS chromatogram and UV₂₆₀ chromatogram) and unmethylated ODN 7 (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the [N⁶mdA-PX] peak.

N-(3-(5-nitropyridin-3-yl)phenyl)hex-5-ynamide

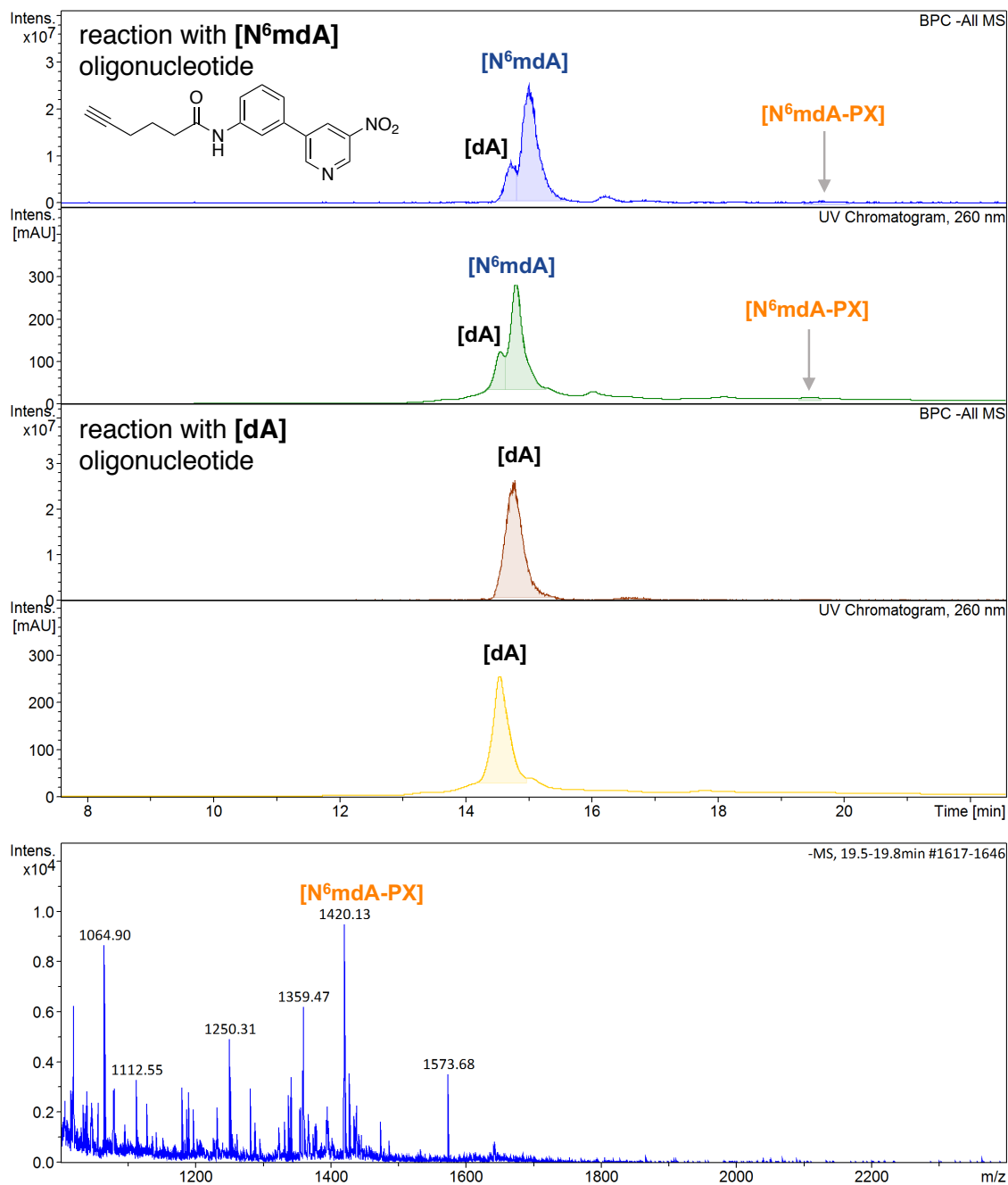


Fig. S10. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with *N*-(3-(5-nitropyridin-2-yl)phenyl)hex-5-ynamide on N^6mdA ODN 5 (top; base peak MS chromatogram and UV₂₆₀ chromatogram) and unmethylated ODN 7 (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the $[N^6mdA-PX]$ peak.

N-(3-(3-nitropyridin-4-yl)phenyl)hex-5-ynamide

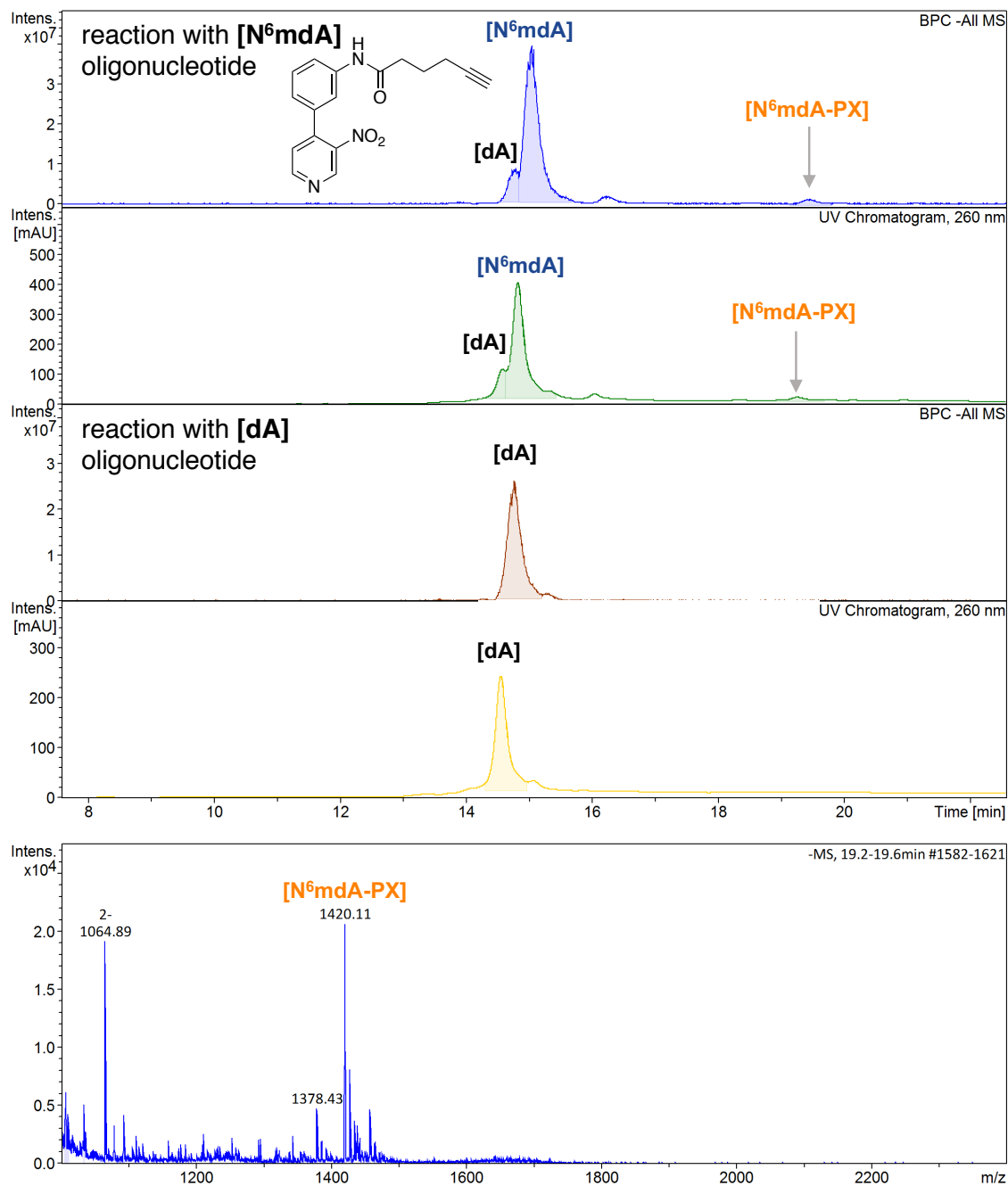


Fig. S11. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with *N*-(3-(3-nitropyridin-4-yl)phenyl)hex-5-ynamide on N^6mdA ODN 5 (top; base peak MS chromatogram and UV₂₆₀ chromatogram) and unmethylated ODN 7 (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the $[N^6mdA-PX]$ peak.

3-(3-nitropyridin-4-yl)-N-(pent-4-yn-1-yl)benzamide

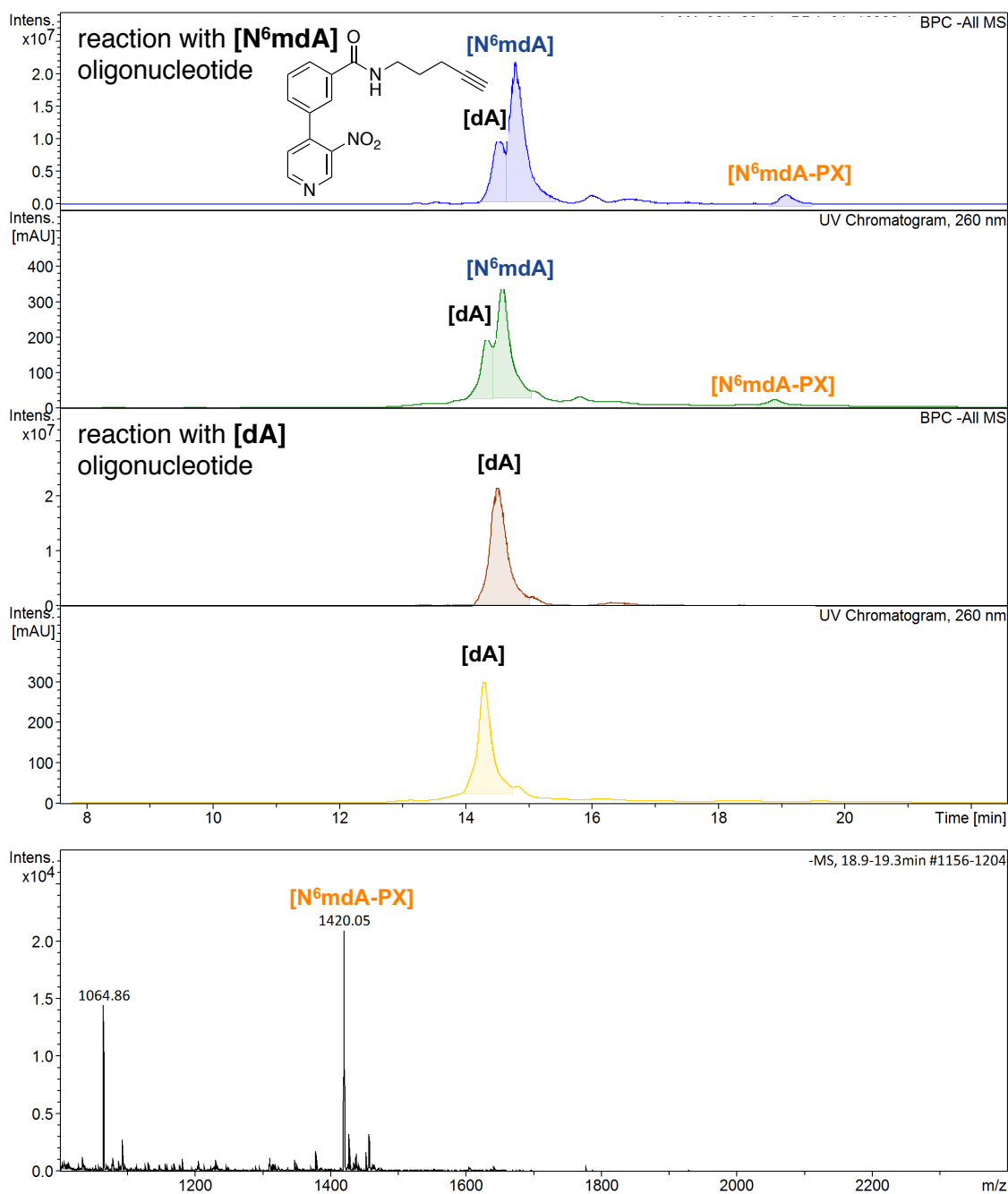


Fig. S12. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with 3-(3-nitropyridin-4-yl)-N-(pent-4-yn-1-yl)benzamide on N^6mdA ODN 5 (top; base peak MS chromatogram and UV₂₆₀ chromatogram) and unmethylated ODN 7 (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the $[N^6mdA-PX]$ peak.

5-nitro-N-(pent-4-yn-1-yl)picolinamide

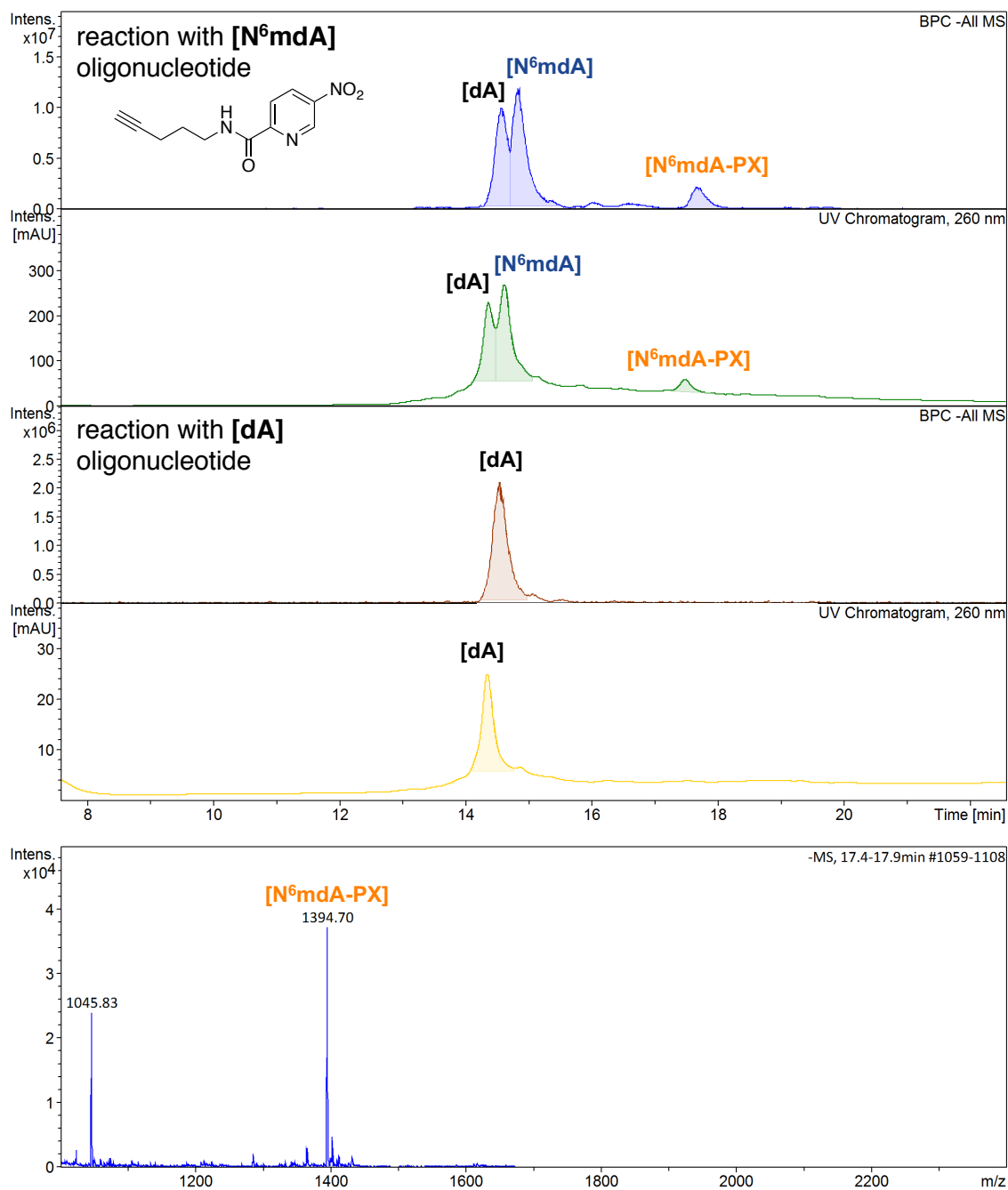


Fig. S13. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with 5-nitro-N-(pent-4-yn-1-yl)picolinamide on N⁶mdA ODN 5 (top; base peak MS chromatogram and UV₂₆₀ chromatogram) and unmethylated ODN 7 (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the [N⁶mdA-PX] peak.

5-nitro-N-(prop-2-yn-1-yl)picolinamide

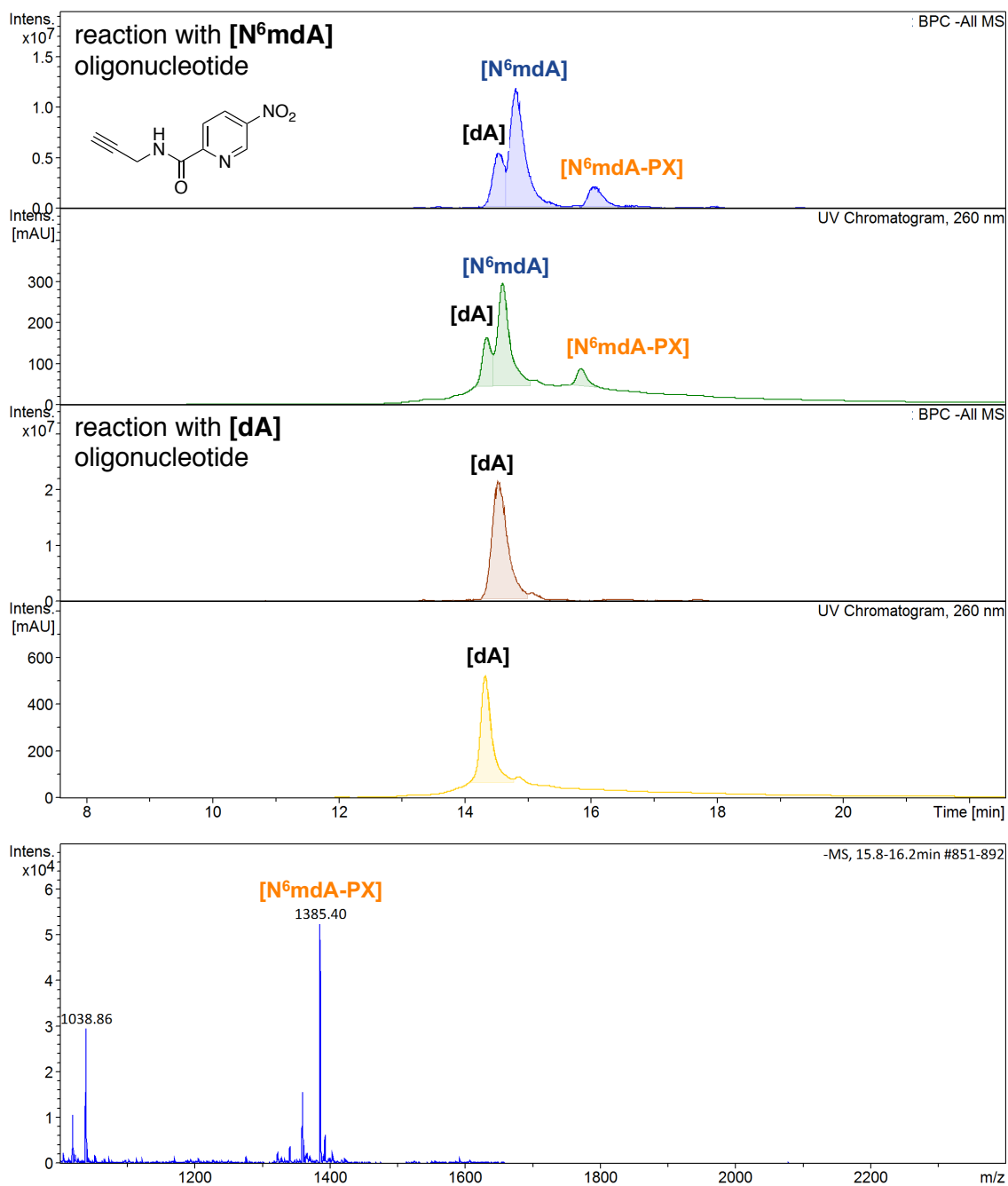


Fig. S15. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with 5-nitro-N-(pent-4-yn-1-yl)picolinamide on N⁶mdA ODN 5 (top; base peak MS chromatogram and UV₂₆₀ chromatogram) and unmethylated ODN 7 (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the [N⁶mdA-PX] peak.

Comparison of photoredox reaction outcome with $[\text{Ru}(\text{bpz})_3](\text{PF}_6)_2$ and $[\text{Ru}(\text{phen})_3]\text{Cl}_2$

Based on the proposed reaction mechanism described in Fig. 2 of the main text, excited $[\text{Ru}(\text{bpz})_3](\text{PF}_6)_2$ undergoes reductive quenching through the oxidation of quinuclidine **3**. Instead, excited $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ directly reduces the 3-nitropyridine through the oxidative quenching cycle. Even though both photocatalysts are able to promote the desired chemistry, which quenching cycle is operative under the reaction conditions could be important for the stability of the starting oligonucleotide and the selectivity of the process. Below, we compared the UV traces of reactions using either $[\text{Ru}(\text{bpz})_3](\text{PF}_6)_2$ or $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ in otherwise identical conditions (Fig. S16).

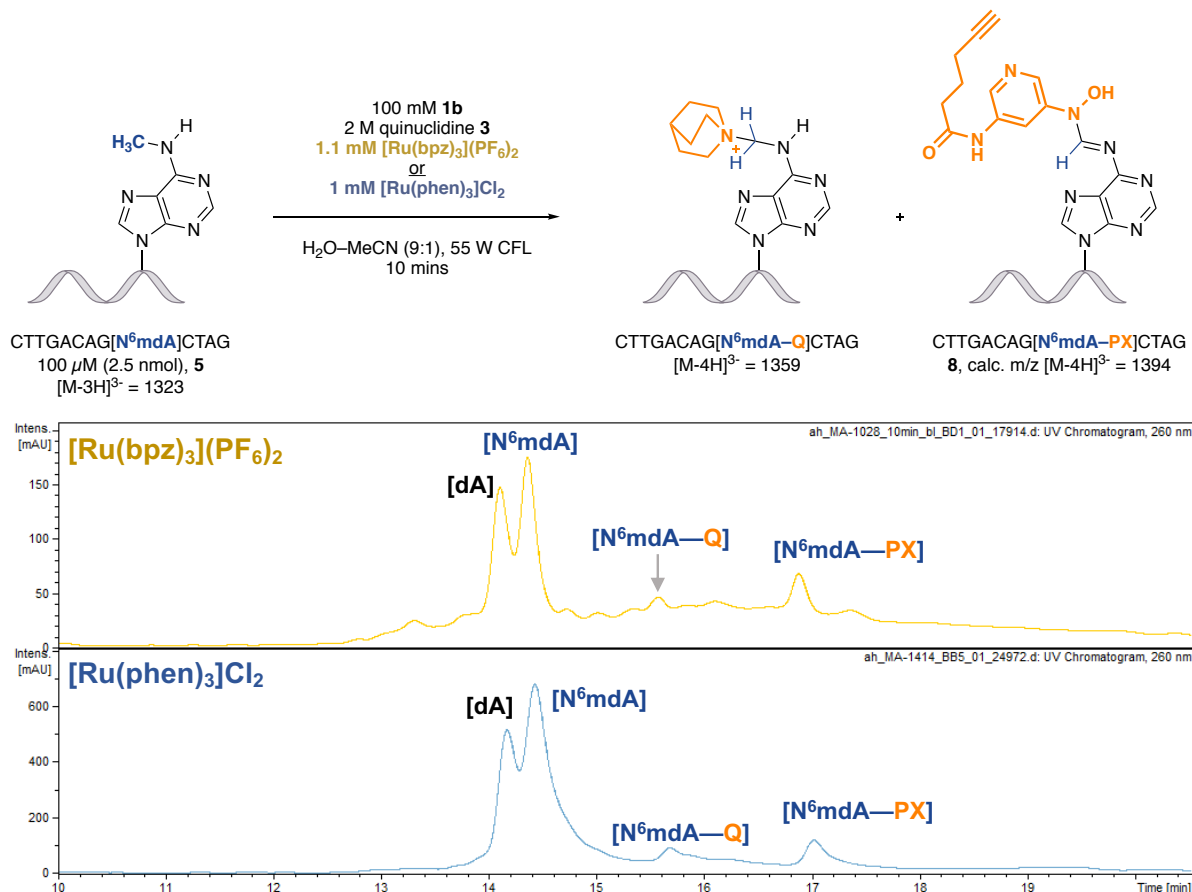


Fig. S16. LCMS traces (UV₂₆₀) of the N^6mdA functionalisation in ODN **5** relying on the reductive quenching pathway of $[\text{Ru}(\text{bpz})_3](\text{PF}_6)_2$ (upper trace, yellow) and the oxidative quenching of $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ (lower trace, blue). According to the UV traces, the use of $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ affords a more selective and clean reaction.

According to the UV traces shown, the use of $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ affords a distinctively more selective and clean reaction, indicating that relying on the oxidative quenching pathway for the generation of the 3-nitrosopyridine derivative and quinuclidinium radical cation to functionalise N^6mdA minimised potential trace side-reactions on the ODN substrate.

HRMS studies on Ru(phen)₃Cl₂ system and photoredox selectivity

Whereas the LCMS traces of the photoredox reaction with [Ru(bpz)₃](PF₆)₂ indicate the occurrence of trace side reactions, we thoroughly analysed the products of the reaction with [Ru(phen)₃]Cl₂ by HRMS to confirm identity of the compounds and understand whether any other 'on-DNA' N-hydroxyformamide product was formed (Fig. S17).

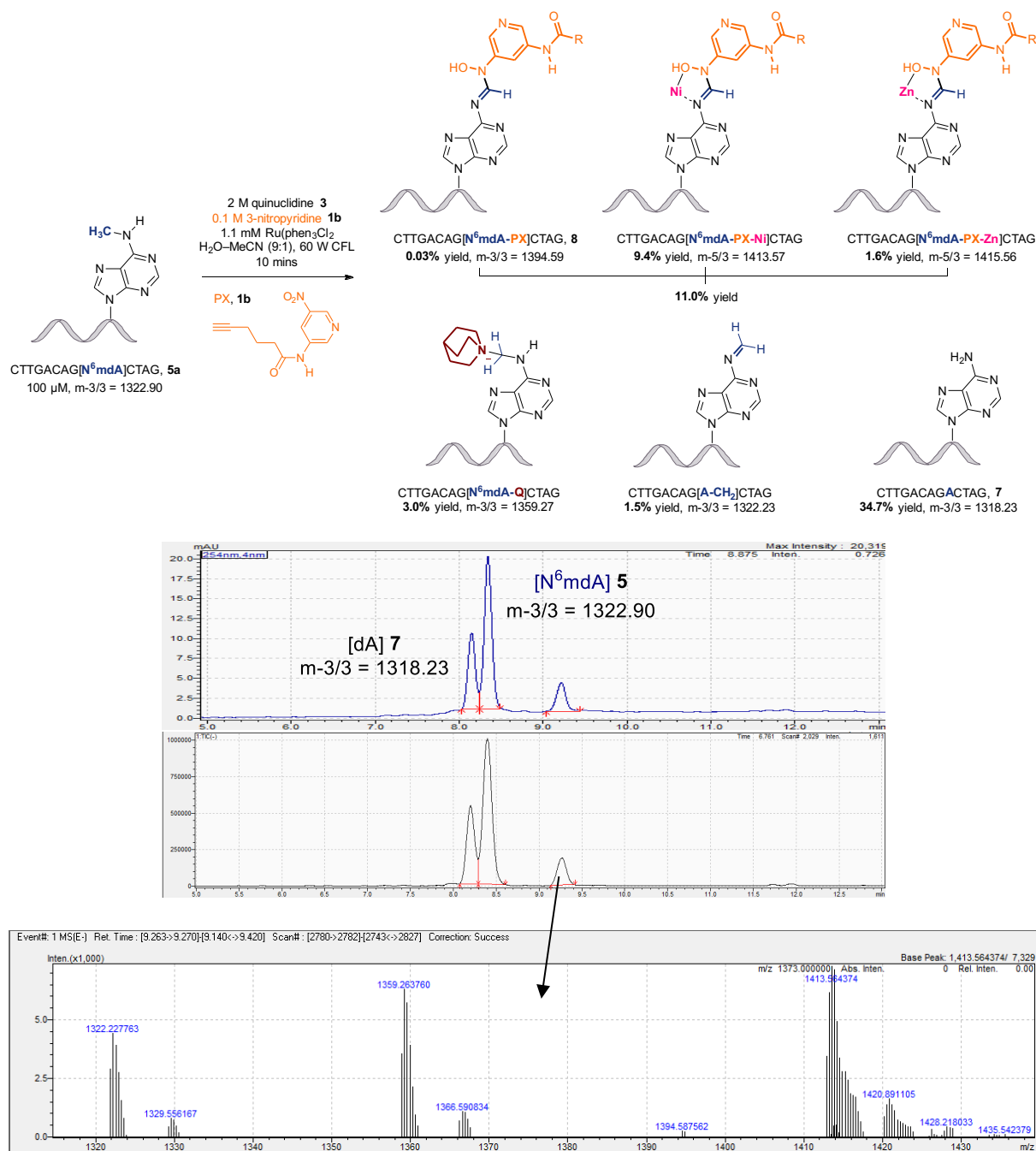


Fig. S17. All the products have been characterized by HMRS using a Shimadzu LC-MS (Q-ToF) 9030. Control experiment using the corresponding oligonucleotide without N⁶mdA did not show any N-hydroxyformamide adducts, searching for the calculated exact masses within a range of 100 ppm. These results indicate >100:1 selectivity (detection limit: 0.00007%) for the HAT process and addition of the α-amino radical to the nitroso intermediate.

Identity of the demethylation product **7**, the quinuclidine derivative [**N⁶mdA-Q**], and the N-hydroxyformamidine product **9** were confirmed by HRMS. We further observed metal complexes of the N-hydroxyformamidine products (with Ni(II) and Zn(II)) as confirmed by the observed high-resolution mass and isotope pattern (Fig. S17). Furthermore, in the reaction mixture from a control reaction with unmethylated ODN **7** as substrate under identical conditions, we could not detect any N-hydroxyformamidine product (with a detection limit of 0.00007%), indicating a >100:1 selectivity of the HAT process and the formation of the N-hydroxyformamidine on **N⁶mdA**. Moreover, HMRS analysis of the photoredox crude reaction mixture showed a peak with the exact mass of the nitroso intermediate **2b** (Pred. C₁₁H₁₁N₃O₂+H = 218.0924, found 218.0926), supporting the proposed mechanism.

HRMS studies on unselective incorporation of intact 3-nitropyridine **1b** at dG residues

To further understand the selectivity of the developed N⁶mdA functionalisation process, we thoroughly analysed the reaction mixture of the photoredox conjugation (according to the scheme in Fig. S17) by HRMS. The only apparent trace products arising from a non-selective reaction were identified as incorporation of the unmodified 3-nitropyridine probe **1b** into the ODN substrate (Fig. S18). No trace of any other side reaction was detected.

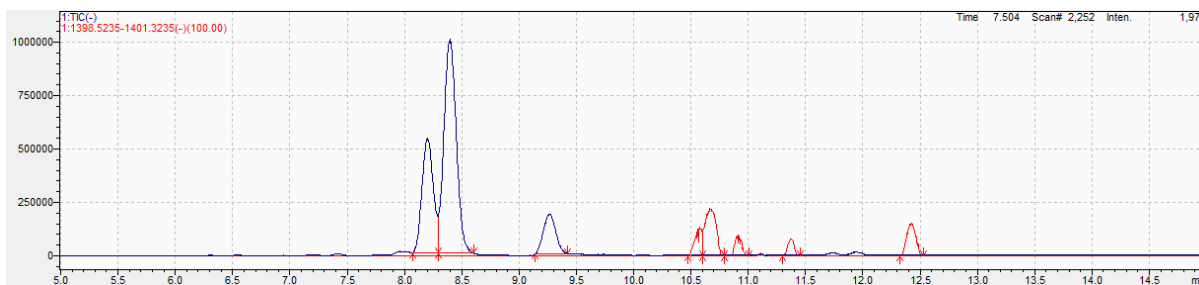


Fig. S18. Non-selective 3-nitropyridine **1b** products (red chromatogram, m-3/3 = 1399.92, 1000 ppm range, magnification factor 100). Incorporation of the unmodified probe (not the nitroso derivative, as in the main pathway resulting in N⁶mdA functionalisation) by a different unknown mechanism.

Importantly, HRMS-based comparison of the trace impurities with the N⁶mdA-specific N-hydroxyformamide formation shows that the overall selectivity for the incorporation of **1b** onto N⁶mdA is 98% (50:1).

To understand if the reaction was specific to one of the canonical deoxynucleosides, we applied the conditions on a set of four unmethylated 13nt ODNs with a 4:4:4:1 distribution of the four canonical nucleosides. Whereas for the ODNs containing only one dA, dC, or dT residue, several products were observed (as for ODN **5** in Fig S18), only two new peaks were found for the ODN containing only one dG residue, indicating that the unselective incorporation of **1b** is specific to dG (Fig. S19).

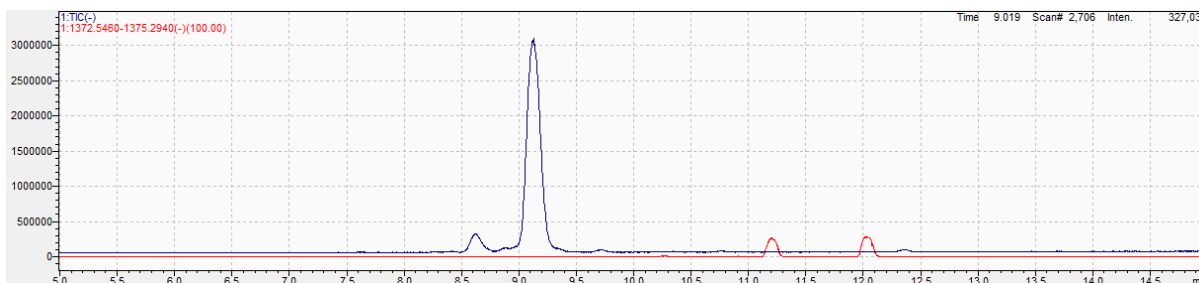


Fig. S19. Non-selective 3-nitropyridine **1b** products (red chromatogram, magnification factor 100) of an oligonucleotide that contains only one dG (ATCTACTGACT). The number of non-selective products decreased in this reaction, suggesting that the trace incorporation of **1b** occurs at dG. Interestingly, the chromatogram shows two distinct non-selective products with 3-nitropyridine **1b**.

Overall, these studies show that the selectivity of N⁶mdA functionalisation with the 3-nitropyridine **1b** versus any other nucleoside is 98% (50:1) and indicate that only dG residues might undergo trace functionalisation, although via a different pathway not resulting in a hydrolysable N-hydroxyformamide linkage.

Collection of evidences for the characterisation of N-hydroxyformamide products

Evidence for the selective functionalisation of the N⁶mdA residue

In the proposed mechanism, we envision the abstraction of an H atom from the N⁶ methyl group of N⁶mdA to obtain the corresponding α -amino radical species (Fig. 2, main text). To confirm the selective HAT and therefore the position of the new label in the observed products, we conducted several control experiments where oligonucleotide **7** (containing the same sequence of oligonucleotide **5** but having dA instead of N⁶mdA) was subjected to our photoredox conditions with various 3-nitropyridine alkyne-derivatives **1** (Fig. S20).

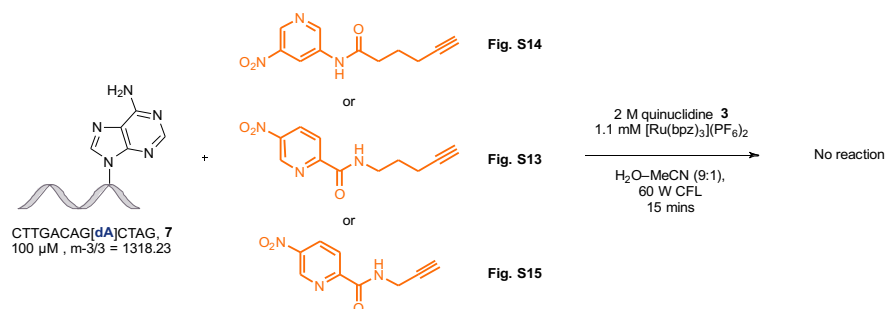


Fig. S20. Control experiments on the unmethylated ODN substrate **7** using different 3-nitropyridine derivatives, confirming that the reaction takes place on the N⁶ methyl group of N⁶mdA.

In all cases, LCMS of the reactions showed recovery of pure unreacted oligonucleotide **7**, with no products apparent (such as the N-hydroxyformamide products when the methylated substrate ODN **5** was used, see Fig. S13, S14 and S15). This confirms that no visible reaction takes place on ODNs without N⁶mdA. **These results not only indicate an exquisite selectivity for the functionalisation of N⁶mdA, but also unambiguously confirm the position of the new label on the N⁶ methyl group (HAT selectivity > 100:1).**

Evidence for the N-hydroxyformamide structural linkage

The N-formamide group follows a distinct hydrolysis process which provides two sets of hydrolysed products containing N-formyl derivatives (*J. Org. Chem.* **1999**, *64*, 991–997). Given this unique feature, we conducted hydrolysis studies on the labelled ODN **8** to confirm the identity of the N-hydroxyformamide structural linkage (Fig. S21). As expected, treatment of the reaction mixture acquired from the photoredox conjugation (containing product **8**) with acetate buffer (pH = 4.7) for 60 minutes fully consumed ODN **8** under formation of three new products: N-formyl ODN **14**, 3-N-hydroxypyridine derivative **15** and 3-N-formyl-N-hydroxypyridine derivative **16**.

Additional evidence for the N-hydroxyformamide linkage are provided by reactivity studies using hydrazine as nucleophile instead of water (Fig. S24).

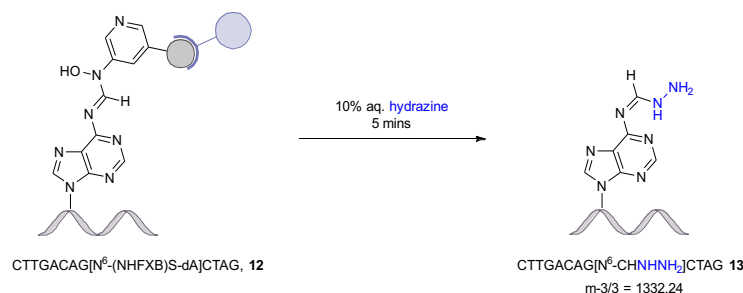


Fig. S24. Reaction of the formed ‘on-DNA’ N-hydroxyformamide with hydrazine to form the N-NH₂ formamide product **13** during pull-down experiments further confirms its identity.

Treatment of immobilised DNA fragment **12** with 10% hydrazine aqueous solution for 5 minutes delivered N-NH₂ formamide ODN **13** along with small amount of **7** (due to the hydrolysis of **13**) as detected by LCMS at the end of the pull-down procedure described in Figure 4 of the main text (see also Fig. S30). **The formation of the hydrazine product 13 upon treatment of the immobilised product 12 is a further clear indication of the identity of the N-hydroxyformamide moiety.**

Another indication of the presence of the N-hydroxyformamide moiety is the detection of Ni(II) and Zn(II) adducts of product **8** and the corresponding click product **10** as confirmed by HRMS and isotopic pattern studies (Fig S25, see also Fig. S17).

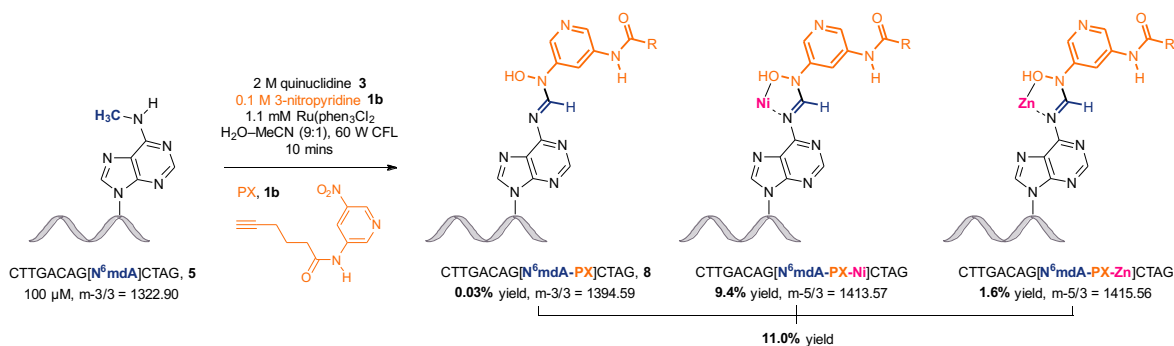


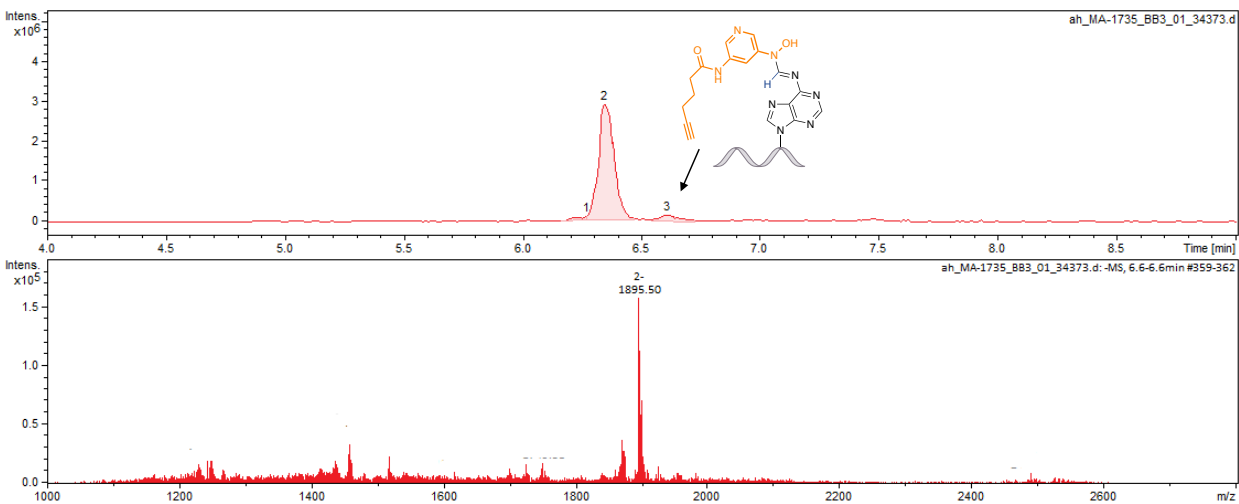
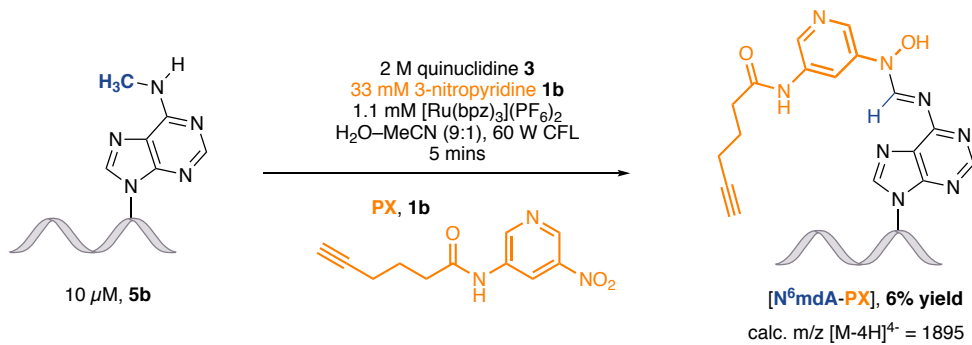
Fig. S25. Formation of Ni(II) and Zn(II) complexes with the ‘on-DNA’ N-hydroxyformamide moiety that acts as a good bidentate for these metals.

Interestingly, Ni(II) and Zn(II) adducts are only visible for the oligonucleotides that contain the N-hydroxyformamide moiety (**8** and **10**). **The formation of stable complexes of the generated N-hydroxyformamides with Ni(II) and Zn(II) is in accordance with previous reports describing these moieties as good bidentate ligands** (*Eur. J. Inorg. Chem.* **2004**, 1740–1752; *Eur. J. Inorg. Chem.* **2016**, 177–185).

Reactions and LC-MS traces for sequences **5b**, **5c**, **5d** and **5e**

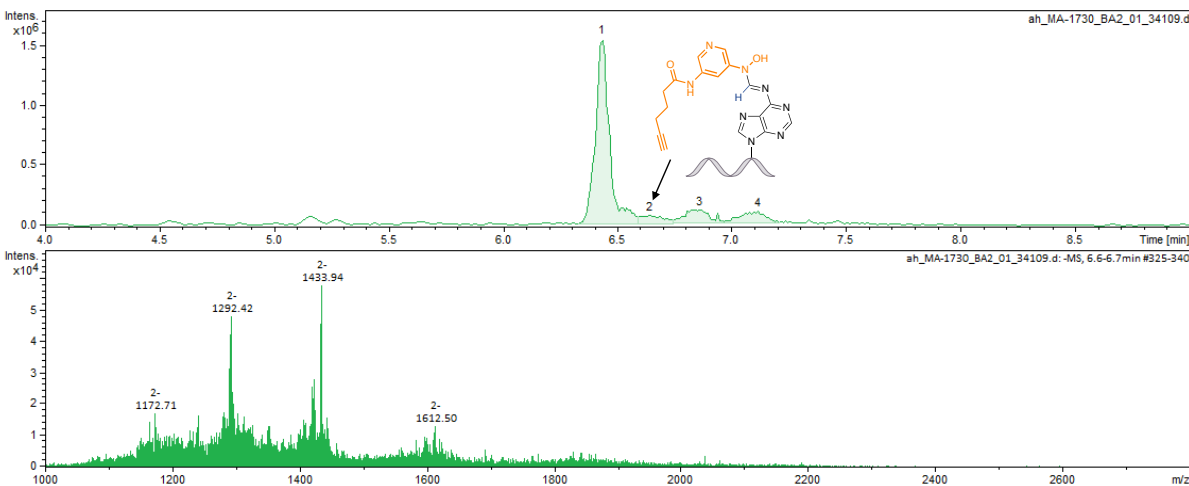
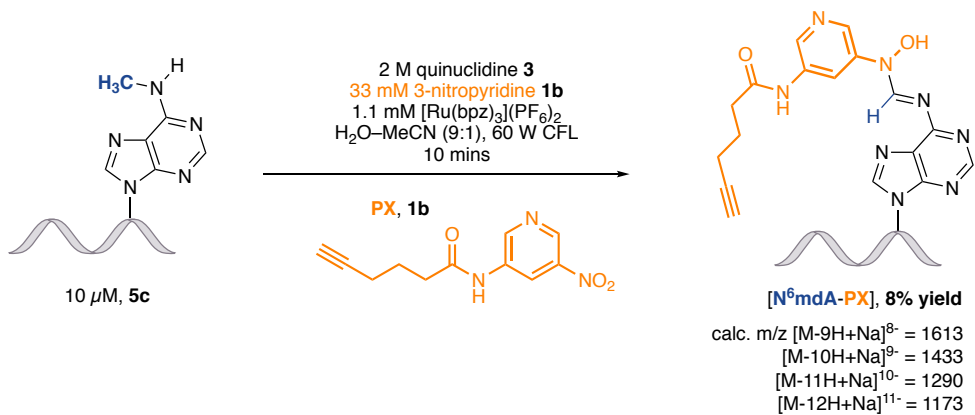
DNA 24mer **5b** sequence (self-complementary):

ATGGCACGTACTAGT [**N⁶mdA**] CGTGCCAT



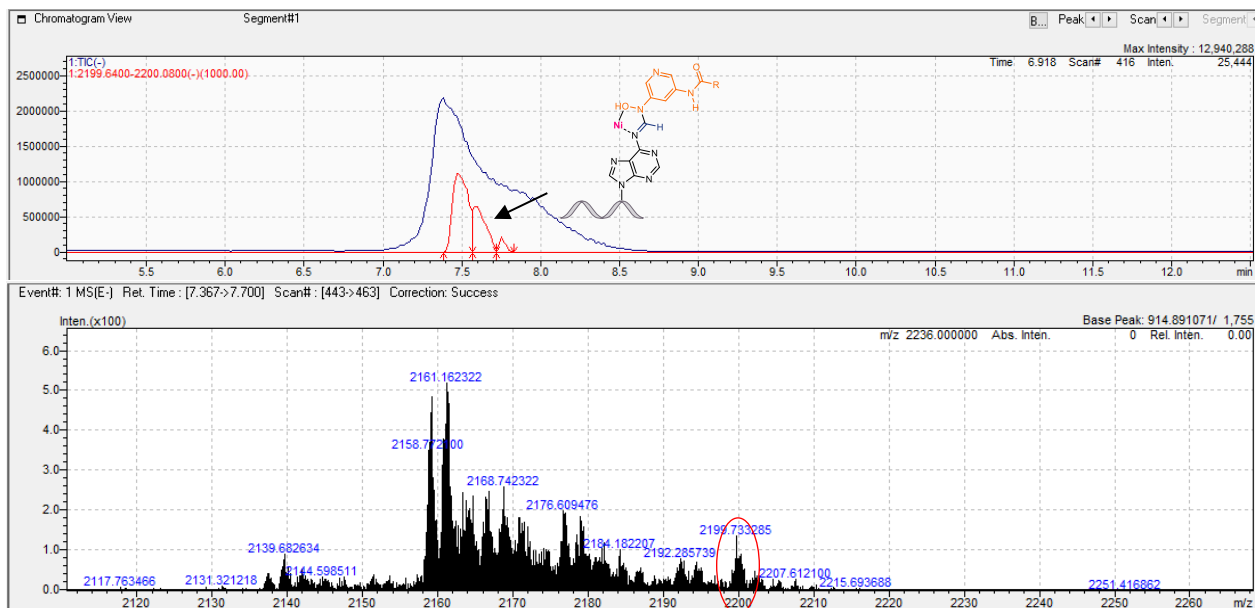
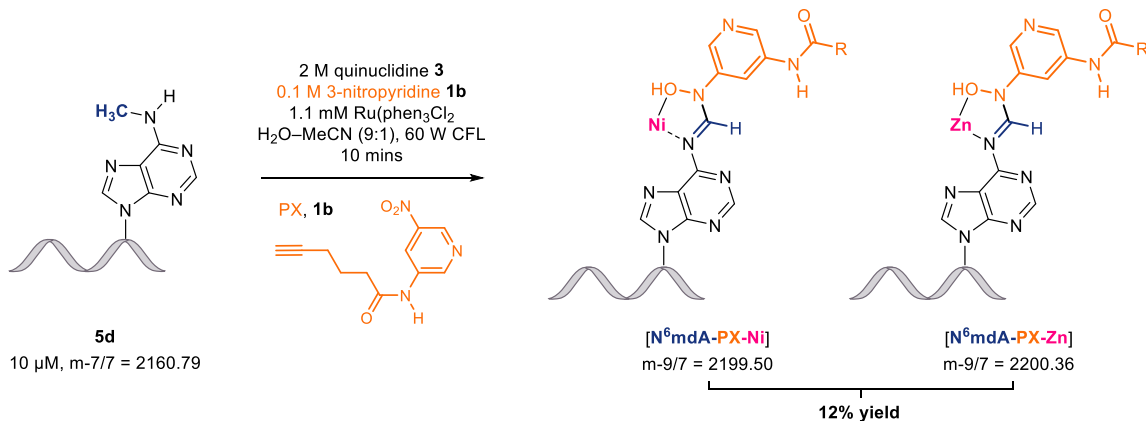
DNA 41mer **5c** sequence:

ATCGTACTAG [^N6mdA] CTCAACGTCTTAGTGGGTGGTCGGTTCCGG

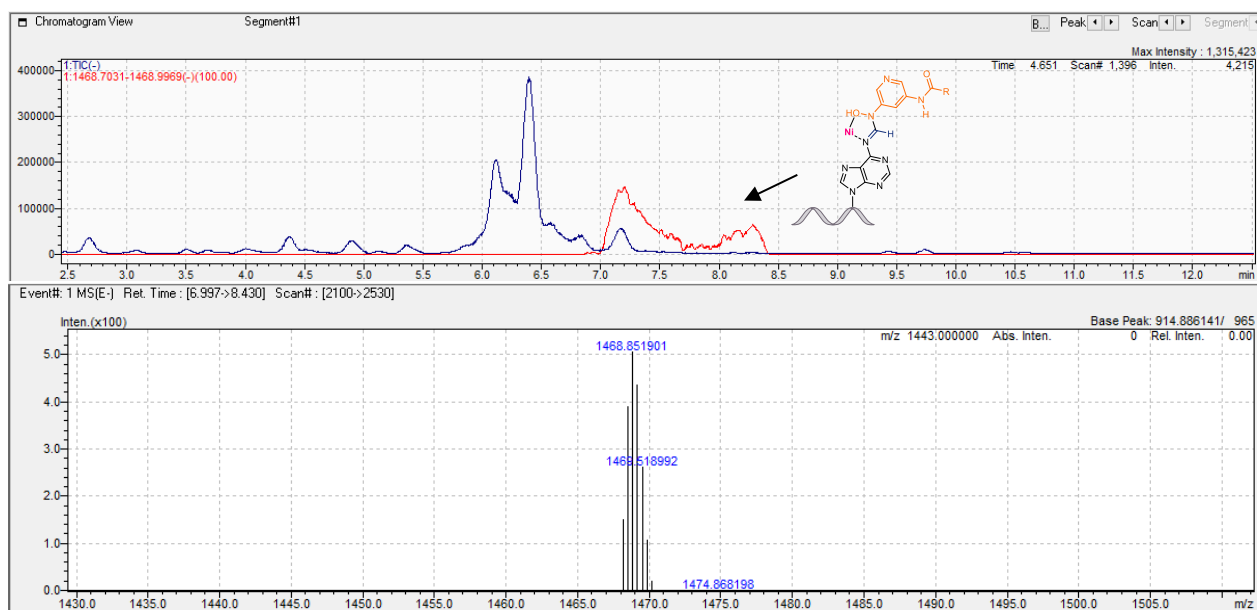
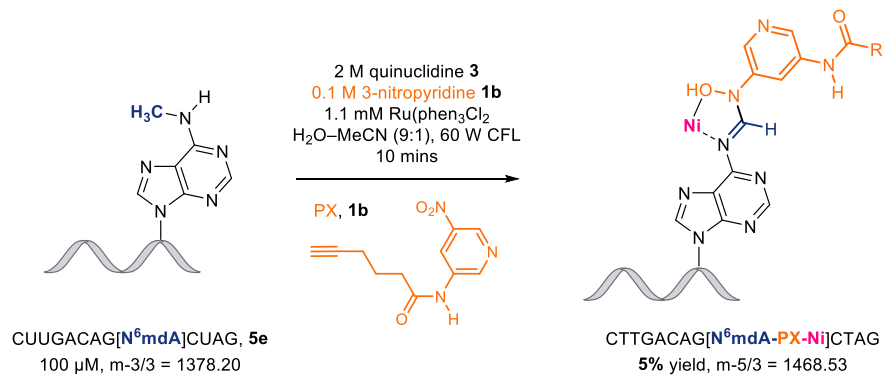


DNA 49mer **5d** sequence:

5' -CTCAGACGTAGCTGATGCTAGGATATCGATCGATGCG [**N⁶mdA**] TCGTAGCTAAC -3'



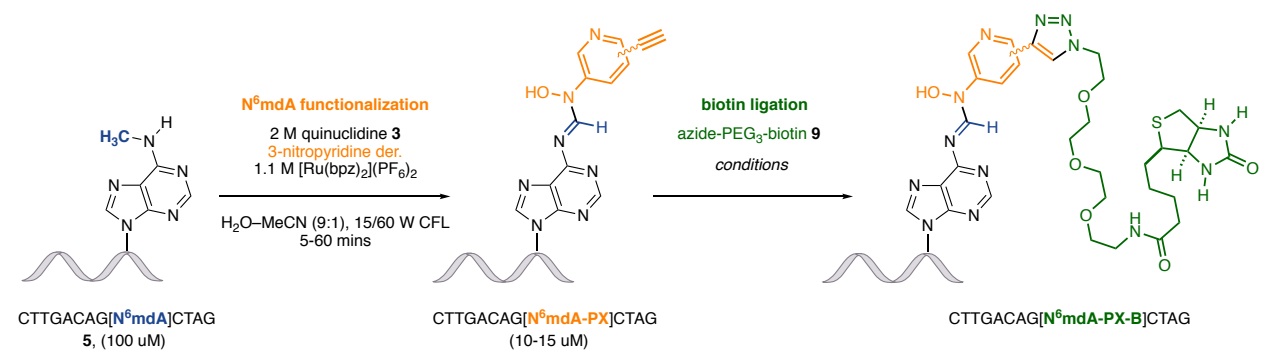
RNA 13mer **5e** sequence:
 5' -CUUGACAG [m⁶A] CUAG-3'



Optimization of ‘click’ Huisgen cycloaddition on alkyne-functionalized N⁶mdA ODN

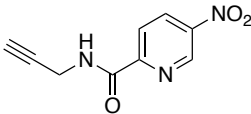
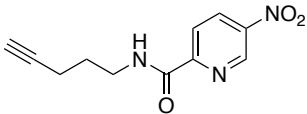
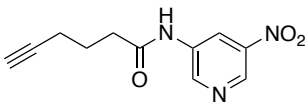
Having demonstrated that several 3-nitropyridine alkyne derivatives are compatible with our N⁶mdA selective photoredox functionalisation, we next evaluated and optimised the reaction conditions for the ‘click’ Huisgen cycloaddition of azide-PEG₃-biotin **9** into various alkyne-functionalized N⁶mdA oligonucleotides. The selective incorporation of a biotin moiety into N⁶mdA residues is fundamental for the next enrichment studies, which rely on the immobilisation of the N⁶mdA DNA fragments into streptavidin-coated magnetic beads.

Table S2. Conditions and yields for the Huisgen cycloaddition of alkyne-functionalized N⁶mdA ODN with azide-PEG₃-biotin **10**. THPTA: Tris(benzyltriazolylmethyl)amine, TCEP: tris(2-carboxyethyl)phosphine, Na-asc.: sodium ascorbate.



3-nitropyridine der.	[c]	photoredox conditions ^a	yield ^b [N ⁶ mdA-P[X]] (m/z [M-3H] ³⁻)	‘click’ conditions	yield ^b [N ⁶ mdA-PX-B] (m/z [M-3H] ³⁻)
	0.04 M	1 h, 15 W CFL	3.5 % (1420)	2 mM 10 0.2 mM CuSO ₄ 1 mM THPTA 0.8 mM TCEP r.t., 1 h	not detected (1568)
	0.04 M	1 h, 15 W CFL	3.5 % (1420)	2 mM 10 0.2 mM CuBr 1 mM THPTA r.t., 1 h	not detected (1568)
	0.04 M	1 h, 15 W CFL	3.5 % (1420)	2 mM 10 0.2 mM CuSO ₄ 1 mM THPTA 0.8 mM Na-asc. r.t., 1 h	23 % (1568)
	0.04 M	1 h, 15 W CFL	3.4 % (1420)	2 mM 10 0.2 mM CuSO ₄ 1 M quinuclidine 0.8 mM Na-asc. r.t., 1 h	24 % (1568)

Table S2 continued

3-nitropyridine der.	[c]	photoredox conditions ^a	yield ^b [N ⁶ mdA-PX] (m/z (M-3H) ³⁻)	'click' conditions	yield ^b [N ⁶ mdA-PX-B] (m/z (M-3H) ³⁻)
	0.04 M	1 h, 15 W CFL	8.0 % ^c (1385)	2 mM 10 0.2 mM CuSO ₄ 1 M quinuclidine 0.8 mM Na-asc. r.t., 1 h	not detected (1533)
	0.05 M	1 h, 15 W CFL	6.6 % (1394)	2 mM 10 0.2 mM CuSO ₄ 1 M quinuclidine 0.8 mM Na-asc. r.t., 30 min	88 % (1542)
	0.05 M	10 min, 60 W CFL	10 % (1394)	2 mM 10 0.2 mM CuSO ₄ 1 M quinuclidine 0.8 mM Na-asc. r.t., 30 min	92 % (1542)

^a other reaction conditions according to General Procedure B

^b assay yields by LCMS

^c [N⁶mdA-Q] species coeluting in LCMS, approximate yields according to MS signal intensities

The results showed that the alkyne substrate, copper ligand, reductant and time are key parameters for the successful outcome of the reaction. To our surprise, quinuclidine proved to be fundamental as copper ligand compare to well-established water-soluble ligands such as Tris((1-benzyl-4-triazolyl)methyl)amine (TBTA) and 3-[tris(3-hydroxypropyltriazolylmethyl)amine] (THPTA). Sodium ascorbate (Na-asc) was superior to tris(2-carboxyethyl)phosphine (TCEP) as reducing agent and 30 minutes was found to be the best time in term of yield.

LCMS traces of functionalization of N⁶mdA ODN **5** with alkyne-derived 3-nitropyridines and subsequent Huisgen cycloaddition

This is a collection of LC traces of the selective photoredox reactions on N⁶mdA containing ODN **5** and subsequent Huisgen cycloaddition described in **Table S2** using different 3-nitropyridine alkyne derivatives (probes). For each probe, the first two chromatograms are the MS and UV traces of the Huisgen cycloaddition using PEG₃ biotin-derived azide **9** on alkyne-functionalized N⁶mdA oligonucleotides (Step 2). The third and the fourth chromatograms represents respectively the MS and UV-trace of the selective photoredox reaction on N⁶mdA containing ODN **5** (Step 1). Finally, the last trace is the MS spectra of the new product peak observed in the Huisgen cycloaddition. The disappearance of the 3-nitropyridine alkyne derivatives [N⁶mdA-PX] obtained after the photoredox conjugation in favour to the new biotin-labelled oligonucleotides [N⁶mdA-PX-B] is a clear evidence for the overall selective biotinylation at N⁶mdA residues.

N-(3-(5-nitropyridin-2-yl)phenyl)hex-5-ynamide

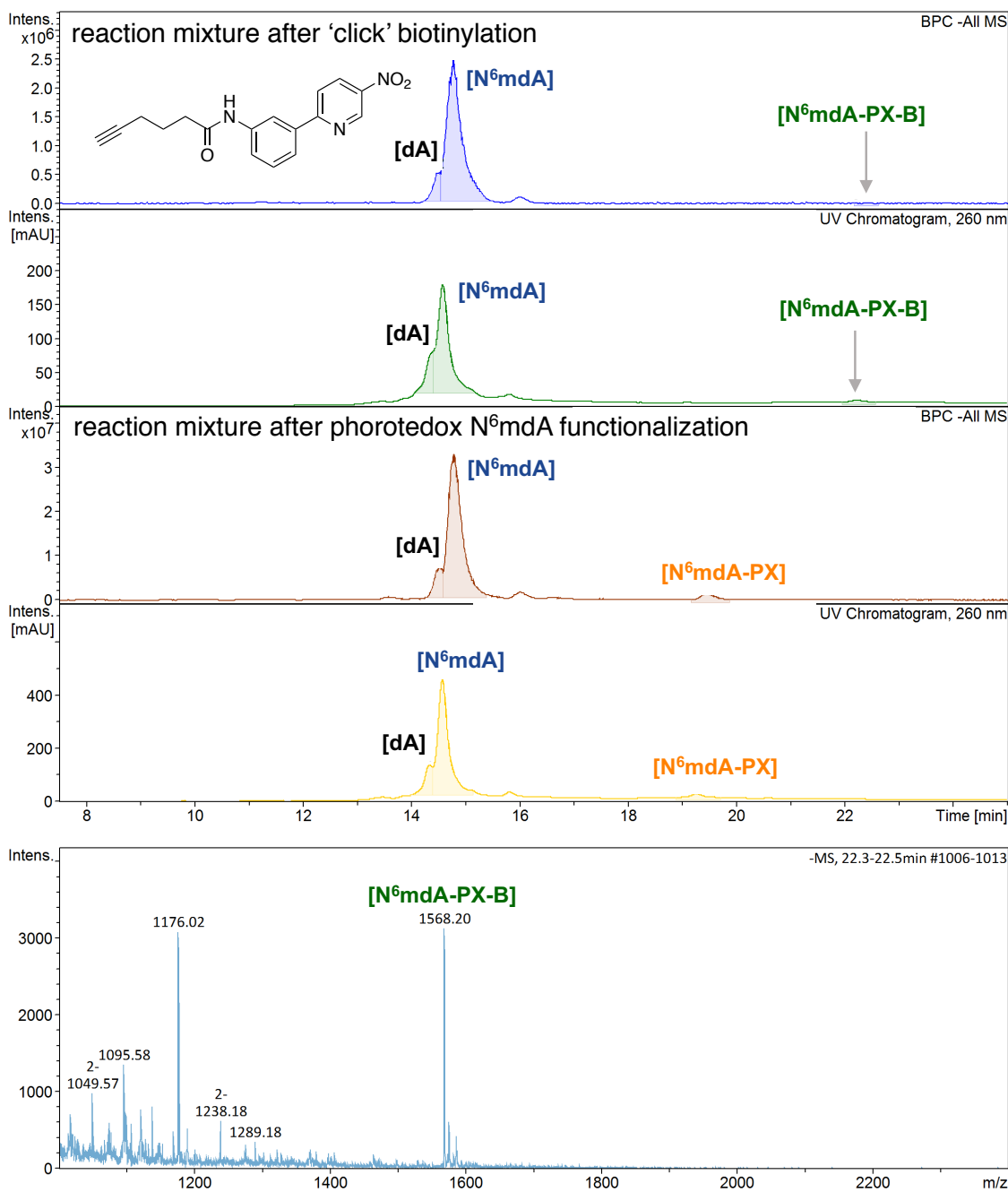


Fig. S26. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with *N*-(3-(5-nitropyridin-2-yl)phenyl)hex-5-ynamide on *N*⁶mdA ODN 5 (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram) and after treatment of the resulting mixture for biotinylation with the optimized Huisgen cycloaddition conditions (top; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the [*N*⁶mdA-PX-B] peak.

5-nitro-N-(pent-4-yn-1-yl)picolinamide

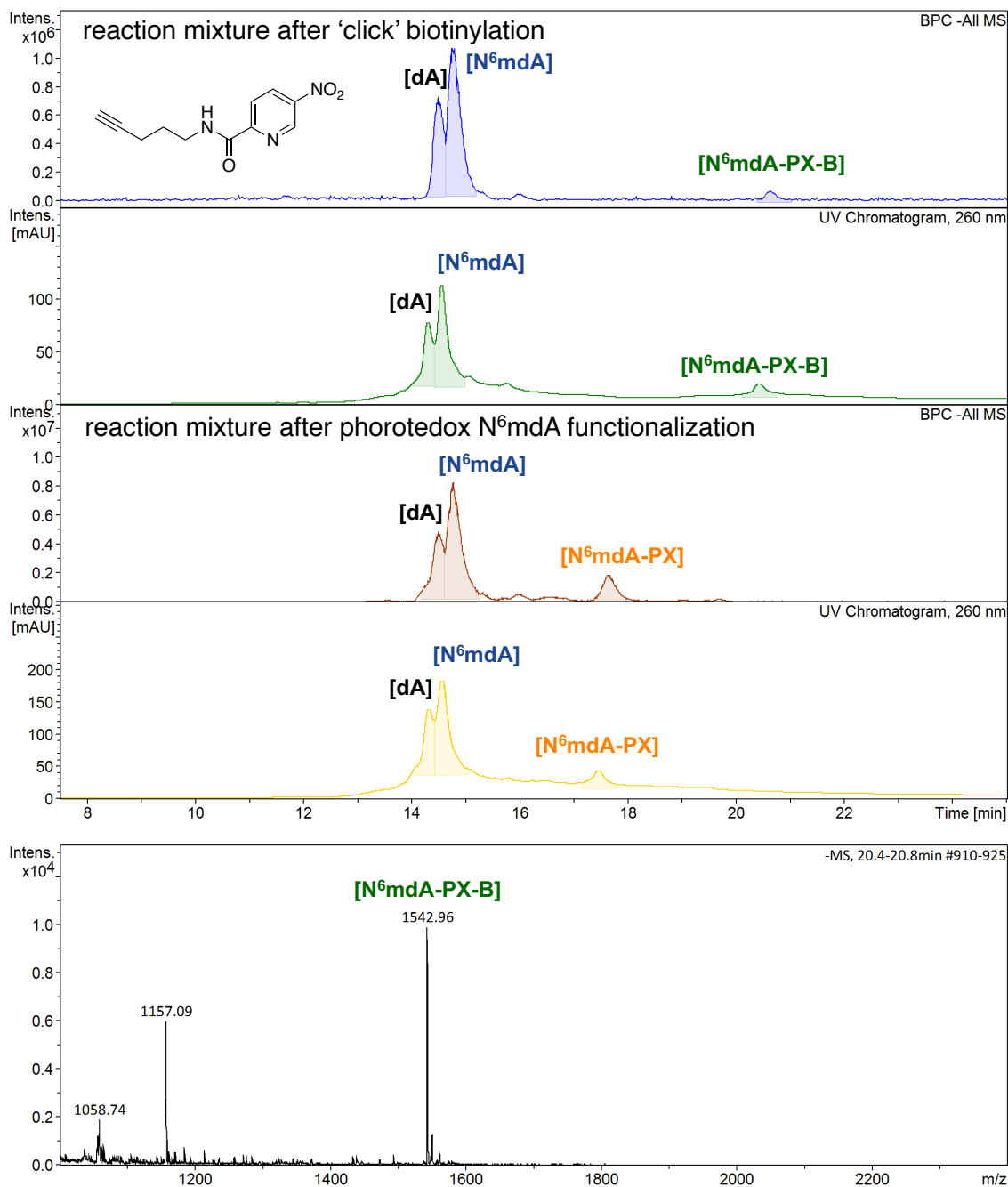


Fig. S27. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with 5-nitro-N-(pent-4-yn-1-yl)picolinamide on N⁶mdA ODN **5** (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram) and after treatment of the resulting mixture for biotinylation with the optimized Huisgen cycloaddition conditions (top; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the [N⁶mdA-PX-B] peak.

N-(5-nitropyridin-3-yl)hex-5-ynamide

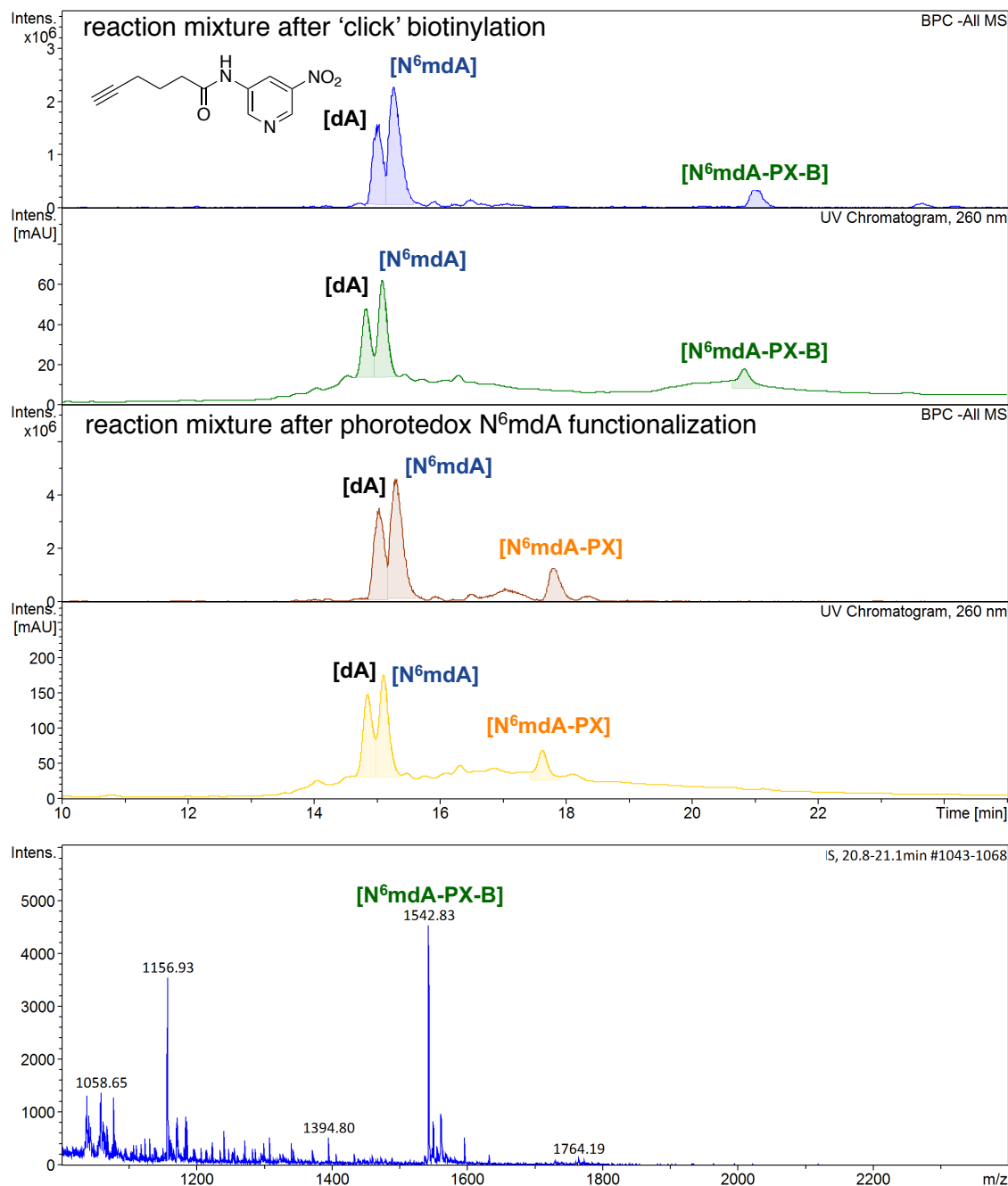


Fig. S28. LCMS traces of the reaction mixtures after applying the photoredox functionalization conditions with *N*-(5-nitropyridin-3-yl)hex-5-ynamide on (bottom; base peak MS chromatogram and UV₂₆₀ chromatogram) and after treatment of the resulting mixture for biotinylation with the optimized Huisgen cycloaddition conditions (top; base peak MS chromatogram and UV₂₆₀ chromatogram). The MS shows the extracted mass spectrum of the [N⁶mdA-PX-B] (10) peak.

LCMS monitoring of 12/13nt ssDNA enrichment experiments

In order to demonstrate the high selectivity of the N⁶mdA functionalisation reaction, we devised an enrichment experiment, consisting of immobilising N⁶mdA-containing ssDNA after biotinylation in the presence of a different unmethylated sequence and washing off unfunctionalised strands. Selective nucleophile-mediated cleavage of the N-hydroxy-formamidinium using an aqueous hydrazine solution afforded an enrichment of >50:1 of the initially methylated ssDNA sequence (Fig. S29).

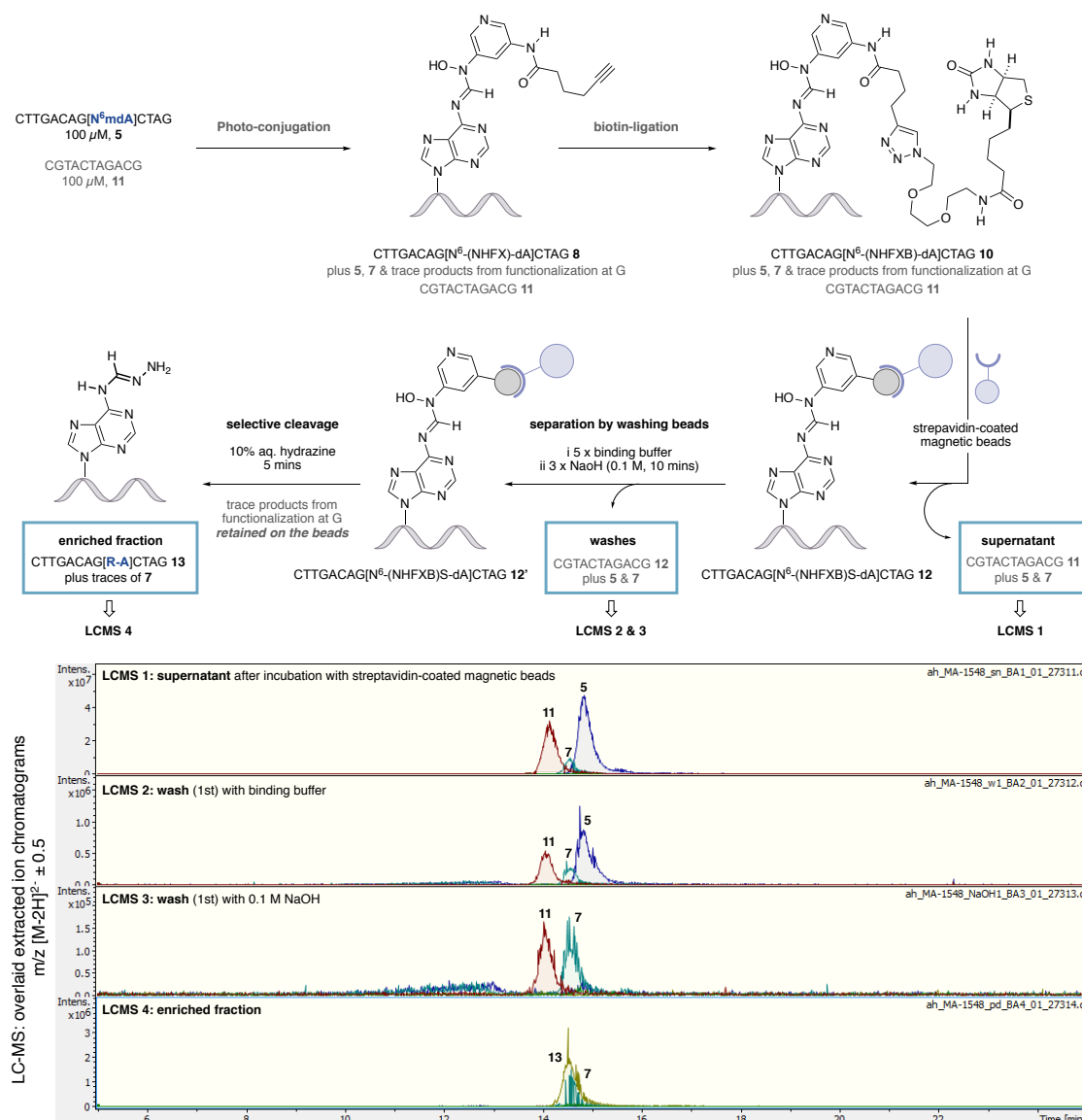


Fig. S29. Detailed procedure of the developed N⁶mdA-specific enrichment procedure with different checkpoints for the LCMS-based analysis of the outcome. After treatment with streptavidin-coated magnetic beads, **LCMS 1** of the supernatant indicates full removal of **10** from the mixture. **LCMS 2** of the first washing mixture shows the three non-biotinylated species **12**, **5** and **7**. After the first incubation with 0.1 M NaOH, residual **12** and **5** were detected (**LCMS 3**). Elution with 10% hydrazine in H₂O releases the selective cleavage product **13** with demethylated **7** and only traces of the other sequence **12**.

To control for sequence effects, we run the same experiment with the N⁶mdA residue being in the other ssDNA sequence (Fig. S30). We obtained an enrichment of >30:1 for the N⁶mdA-containing ssDNA sequence, confirming that the enrichment was N⁶mdA-specific.

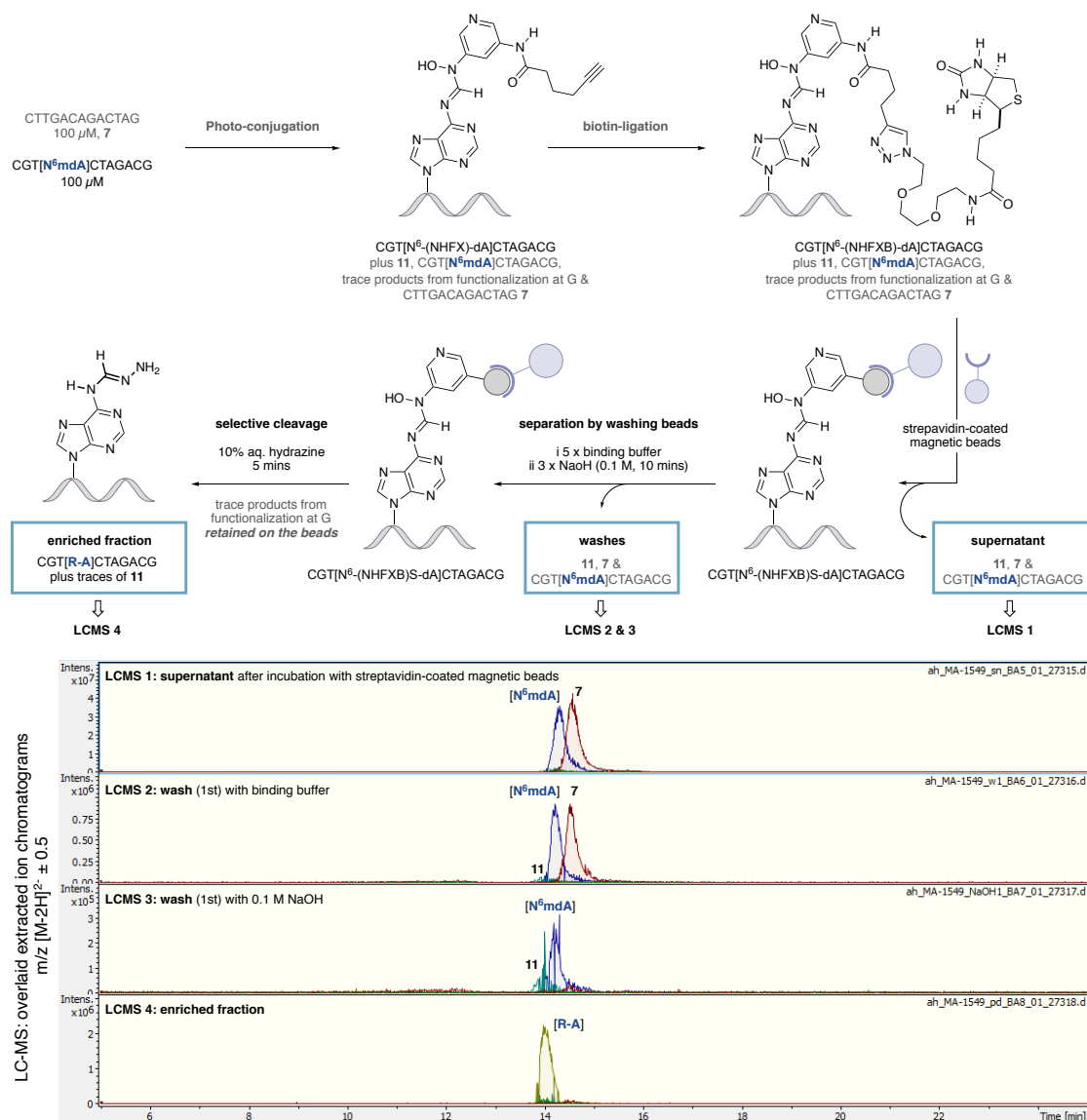


Fig. S30. Detailed procedure of the control of the N⁶mdA-specific enrichment procedure with an inverted set of ssDNA sequences. After treatment with streptavidin-coated magnetic beads, **LCMS 1** of the supernatant indicates full removal of [N⁶-(NHFXB)-dA] from the mixture. **LCMS 2** of the first washing mixture shows the three non-biotinylated species 7, [N⁶mdA] and low amounts of 11. After the first incubation with 0.1 M NaOH, residual 12 and 5 were detected (**LCMS 3**). Elution with 10% hydrazine in H₂O releases the selective cleavage product [R-A] with demethylated 11 and only traces of the other sequence 7.

Enrichment studies of 99nt ssDNA

To test if the developed N⁶mdA-specific DNA enrichment protocol can also be applied on longer ssDNA strands, and if enriched DNA can be amplified, we applied it on a set of two 99nt ssDNA strands (with an N⁶mdA residue being in one sequence for (a) and in the other for (b)) and quantified both sequences by qPCR after enrichment (Fig. S31). Obtained enrichment factors of 6:1 for the respective methylated sequences demonstrated the applicability of the enrichment procedure on 99nt DNA strands.

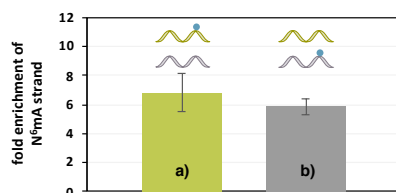
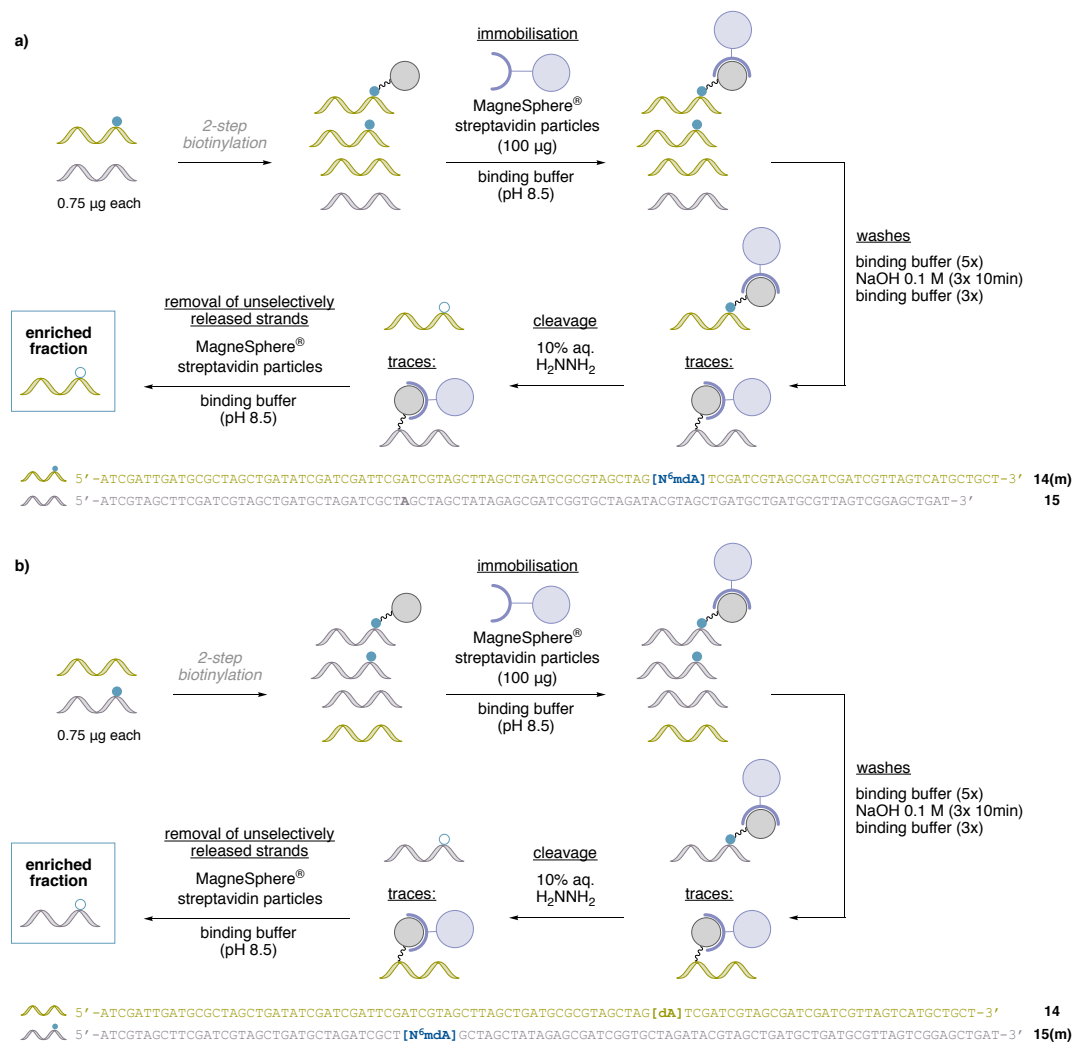


Fig. S31. Top: Scheme of the enrichment experiments with a set of two synthetic 99nt ssDNA strands with either one (a) or the other (b) being methylated. Bottom: outcome for the enrichment experiments (fold enrichment of amplifiable N⁶mdA-containing strand) as determined by qPCR. Mean values of two independent experiments, error bars indicate standard deviation.

Synthesis of compounds and oligonucleotides

Synthesis and characterization of derivatives of quinuclidine and 3-nitropyridine

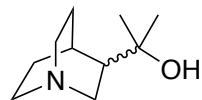
General procedure J: Suzuki coupling of aryl boronic acids to chloro- or bromo-3-nitropyridines

The appropriate chloro- or bromo- 3-nitropyridine and arylboronic acid were dissolved in a 1:1 (v/v) mixture of THF and 20 % (wt) Na₂CO₃ in H₂O under an atmosphere of argon. Tetrakis(triphenylphosphine)palladium(0) (10-20 mol%) was added and the mixture was refluxed for 2 h. After dilution with H₂O the mixture was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (MgSO₄) and the solvents evaporated. Crude products were purified by flash column chromatography.

General procedure K: Amide couplings of aromatic amines or carboxylic acids with 5-hexynoic acid or propargylamine/4-pentyne-1-amine

The appropriate carboxylic acid (1 equiv.), amine (1 equiv.) and Et₃N (1 equiv.) were dissolved in dry DMF under an atmosphere of argon. The mixture was cooled to 0 °C and pyBOP (1 equiv.) was added. The mixture was stirred overnight, allowing it to slowly reach r.t. It was then diluted with H₂O and extracted with EtOAc. The combined organic layers were dried (MgSO₄) and the solvents evaporated. Crude products were purified by flash column chromatography.

2-(quinuclidin-3-yl)propan-2-ol

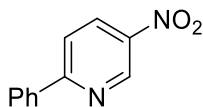


Methyl-3-quinuclidinecarboxylate hydrochloride was converted to its free base by dissolution in sat. aq. K₂CO₃ and extraction with CHCl₃.

Methyl-3-quinuclidinecarboxylate (99 mg, 0.59 mmol) was dissolved in 5 ml dry Et₂O under an atmosphere of argon and cooled to -78 °C. MeLi (1.6 M in Et₂O, 1.5 ml, 2.36 mmol) was added slowly and the mixture was stirred at -78 °C for 3 h. The reaction was quenched with sat. aq. NH₄Cl (0.2 ml), and sat. aq. K₂CO₃ (5 ml) was subsequently added. This mixture was extracted with Et₂O, the combined organic layers were dried with MgSO₄, and the solvents were evaporated. The crude product was purified by flash column chromatography (0-10% MeOH in CH₂Cl₂ with 0.5 % Et₃N). The purified product was extracted from sat. aq. K₂CO₃ (5 ml) with Et₂O to afford free base of 2-(quinuclidin-3-yl)propan-2-ol (30 mg, 0.18 mmol, 31%).

¹H NMR (400 MHz, Methanol-*d*₄) δ (ppm) 3.01 – 2.66 (m, 6H), 2.20 – 2.09 (m, 1H), 1.99 (s, 1H), 1.73 – 1.53 (m, 3H), 1.44 – 1.32 (m, 1H), 1.23 (s, 3H), 1.17 (s, 3H). ¹³C NMR (101 MHz, Methanol-*d*₄) δ 72.9, 50.5, 48.2, 47.4, 46.7, 30.5, 28.8, 28.6, 23.5, 23.3. HMRS-ESI (m/z): found [M+H]⁺ 170.1537 C₁₀H₂₀NO requires 170.1545

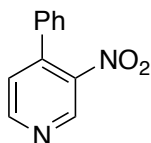
5-nitro-2-phenylpyridine



Synthesised according to General Procedure J from 2-bromo-5-nitropyridine (203 mg, 1 mmol) and phenylboronic acid (183 mg, 1.5 mmol) to afford product as a white solid (112 mg, 0.56 mmol, 56%).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.50 (dd, *J* = 2.7, 0.6 Hz, 1H), 8.53 (dd, *J* = 8.8, 2.7 Hz, 1H), 8.13 – 8.06 (m, 2H), 7.92 (dd, *J* = 8.8, 0.6 Hz, 1H), 7.57 – 7.51 (m, 3H); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) 162.6, 145.4, 143.0, 137.2, 132.1, 131.0, 129.3, 127.8, 120.2; **HMRS-ESI** (m/z): found [M+H]⁺ 201.0660, C₁₁H₉N₂O₂ requires 201.0659.

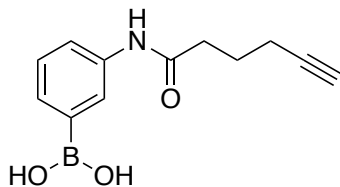
3-nitro-4-phenylpyridine



Synthesised according to General Procedure J from 4-chloro-3-nitropyridine (1.00 g, 6.3 mmol) and phenylboronic acid (0.90 g, 7.4 mmol) to afford product as a pale yellow solid (0.65 g, 3.3 mmol, 52%).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.08 (s, 1H), 8.81 (d, *J* = 5.1 Hz, 1H), 7.50 – 7.46 (m, 3H), 7.42 (d, *J* = 5.0 Hz, 1H), 7.37 – 7.33 (m, 2H). **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) 152.8, 145.7, 145.3, 144.1, 134.56, 129.8, 129.2, 127.7, 125.9. **HMRS-ESI** (m/z): found [M+H]⁺ 201.0656, C₁₁H₉N₂O₂ requires 201.0659.

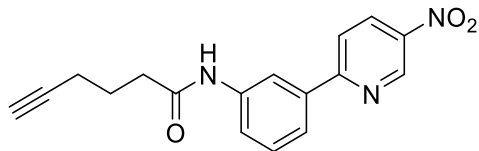
(3-(hex-5-ynamido)phenyl)boronic acid



Synthesised according to General Procedure K from 3-aminophenylboronic acid monohydrate (310 mg, 2.0 mmol) and 5-hexynoic acid (0.22 ml, 2.0 mmol) to afford the product as a white solid containing 15mol% tripyrrolidinophosphine oxide (344 mg, 1.3 mmol, 67 %) which was used without further purification for subsequent suzuki couplings.

¹H NMR (400 MHz, Methanol-*d*₄) δ (ppm) 7.84 – 7.72 (m, 1H), 7.61 (d, *J* = 7.3 Hz, 1H), 7.51 – 7.22 (m, 2H), 3.14 (td, *J* = 6.6, 3.7 Hz, 2H), 2.50 (t, *J* = 7.5 Hz, 2H), 2.32 – 2.23 (m, 1H), 1.88 (p, *J* = 7.3 Hz, 2H). **¹³C NMR** (101 MHz, Methanol-*d*₄) δ (ppm) 173.8, 139.2, 130.2, 129.1, 126.2, 122.6, 84.1, 70.3, 36.6, 25.7, 18.7 (one quaternary aromatic C not detected). **HMRS-ESI** (m/z): found [M+H]⁺ 232.1135, C₁₂H₁₅BNO₃ requires 232.1140.

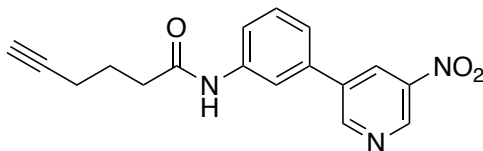
N-(3-(5-nitropyridin-2-yl)phenyl)hex-5-ynamide



Synthesised according to General Procedure J from 2-bromo-5-nitropyridine (102 mg, 0.5 mmol) and (3-(hex-5-ynamido)phenyl)boronic acid (116 mg, 0.5 mmol) to afford the product as a pale yellow solid (52 mg, 0.17 mmol, 34%).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.48 (dd, *J* = 2.7, 0.6 Hz, 1H), 8.53 (dd, *J* = 8.8, 2.7 Hz, 1H), 8.35 (app s, 1H), 7.94 (dd, *J* = 8.8, 0.6 Hz, 1H), 7.83 (app d, *J* = 8.0 Hz, 1H), 7.68 (app d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.42 – 7.32 (bs, 1H), 2.57 (t, *J* = 7.3 Hz, 2H), 2.36 (td, *J* = 6.8, 2.6 Hz, 2H), 2.04 (t, *J* = 2.6 Hz, 1H), 1.99 (qnt, *J* = 7.1 Hz, 2H); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) 170.9, 162.0, 145.3, 143.2, 138.9, 138.0, 132.1, 130.0, 123.6, 122.1, 120.4, 119.0, 83.5, 70.0, 36.1, 24.0, 17.9; **HMRS-ESI** (m/z): found [M+H]⁺ 310.1188, C₁₇H₁₆N₃O₃ requires 310.1186.

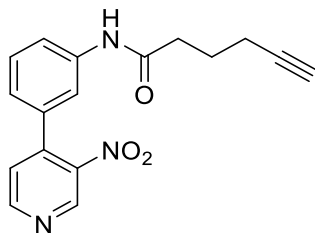
N-(3-(5-nitropyridin-3-yl)phenyl)hex-5-ynamide



Synthesised according to General Procedure J from 3-bromo-5-nitropyridine (51 mg, 0.25 mmol) and (3-(hex-5-ynamido)phenyl)boronic acid (53 mg, 0.23 mmol) to afford the product as a pale orange solid (46 mg, 0.15 mmol, 65%).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.42 (d, *J* = 2.4 Hz, 1H), 9.13 (d, *J* = 2.0 Hz, 1H), 8.66 (app. t, *J* = 2.3 Hz, 1H), 8.01 (app. s, 1H), 7.52 – 7.42 (m, 3H), 7.37 (d, *J* = 7.0 Hz, 1H), 2.58 (t, *J* = 7.3 Hz, 2H), 2.36 (td, *J* = 6.7, 2.6 Hz, 2H), 2.03 (t, *J* = 2.6 Hz, 1H), 1.97 (app. q, *J* = 7.0 Hz, 2H). **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) 171.0, 153.2, 144.6, 143.6, 139.1, 137.4, 136.2, 130.3, 129.3, 123.2, 120.5, 118.7, 83.5, 69.7, 36.1, 23.9, 17.9. **HMRS-ESI** (*m/z*): found [M+H]⁺ 310.1181, C₁₇H₁₆N₃O₃ requires 310.1186.

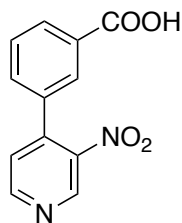
N-(3-(3-nitropyridin-4-yl)phenyl)hex-5-ynamide



Synthesised according to General Procedure J from 4-chloro-3-nitropyridine (150 mg, 0.95 mmol) and (3-(hex-5-ynamido)phenyl)boronic acid (200 mg, 0.87 mmol) to afford the product as an off-white solid (187 mg, 0.61 mmol, 70%).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.08 (s, 1H), 8.81 (d, *J* = 5.0 Hz, 1H), 7.70 (app s, 1H), 7.51 (app d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 5.8 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.36 – 7.31 (bs, 1H), 7.07 (app d, *J* = 7.8 Hz, 1H), 2.54 (t, *J* = 7.3 Hz, 2H), 2.34 (td, *J* = 6.8, 2.6 Hz, 2H), 2.02 (t, *J* = 2.6 Hz, 1H), 1.96 (qnt, *J* = 7.0 Hz, 2H); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) 170.7, 152.9, 145.5, 145.2, 134.5, 129.7, 125.8, 123.4, 120.6, 118.9, 83.3, 69.6, 35.9, 23.7, 17.8; **HMRS-ESI** (*m/z*): found [M+H]⁺ 310.1189, C₁₇H₁₆N₃O₃ requires 310.1186.

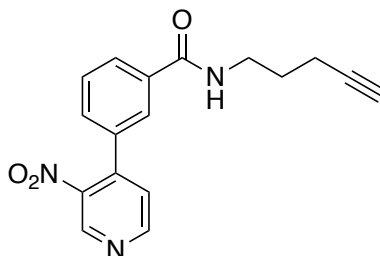
3-(3-nitropyridin-4-yl)benzoic acid



Synthesised according to General Procedure J from 4-chloro-3-nitropyridine (200 mg, 1.26 mmol) and 3-carboxyphenylboronic acid (231 mg, 1.39 mmol) with extended reaction time (reflux for 20 h) to afford the product as a pale orange solid (162 mg, 0.60 mmol, 47%).

¹H NMR (500 MHz, CDCl₃) δ (ppm) 9.19 (s, 1H), 8.88 (d, *J* = 5.0 Hz, 1H), 8.23 (d, *J* = 7.5 Hz, 1H), 8.11 (s, 1H), 7.61 (app. t, *J* = 7.5 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.45 (d, *J* = 4.9 Hz, 1H). **¹³C NMR** (126 MHz, CDCl₃) δ 168.9, 153.1, 145.6, 145.1, 143.1, 135.2, 132.7, 131.2, 130.1, 129.4, 129.3, 125.8. **HMRS-ESI** (m/z): found [M+H]⁺ 245.0551, C₁₂H₉N₂O₄ requires 245.0557.

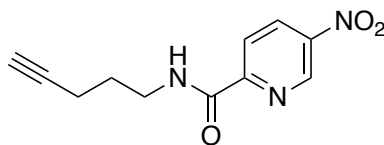
3-(3-nitropyridin-4-yl)-N-(pent-4-yn-1-yl)benzamide



Synthesised according to General Procedure K from 3-(3-nitropyridin-4-yl)benzoic acid (18 mg, 0.074 mmol) and 4-pentyne-1-amine hydrochloride (11 mg, 0.089 mmol) to afford the product as an off-white solid (17 mg, 0.055 mmol, 74 %).

¹H NMR (500 MHz, CDCl₃) δ (ppm) 9.15 (s, 1H), 8.85 (d, *J* = 5.0 Hz, 1H), 7.83 (ddd, *J* = 7.7, 1.8, 1.1 Hz, 1H), 7.80 (dt, *J* = 1.8, 0.9 Hz, 1H), 7.54 (td, *J* = 7.7, 0.6 Hz, 1H), 7.46 – 7.41 (m, 2H), 6.47 (app. s, 1H), 3.61 (td, *J* = 6.7, 5.8 Hz, 2H), 2.34 (td, *J* = 6.8, 2.7 Hz, 2H), 2.03 (t, *J* = 2.7 Hz, 1H), 1.88 (app. p, *J* = 6.8 Hz, 2H). **¹³C NMR** (126 MHz, CDCl₃) δ 166.8, 153.2, 145.6, 145.3, 143.5, 135.8, 135.4, 130.6, 129.4, 127.7, 126.7, 126.0, 83.76, 69.7, 39.7, 28.0, 16.5. **HMRS-ESI** (m/z): found [M+H]⁺ 310.1182, C₁₇H₁₆N₃O₃ requires 310.1186.

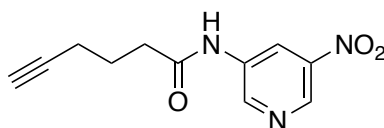
5-nitro-N-(pent-4-yn-1-yl)picolinamide



Synthesised according to General Procedure K from 5-nitropyridine-2-carboxylic acid (141 mg, 0.84 mmol) and 4-pentyne-1-amine hydrochloride (100 mg, 0.84 mmol) to afford the product as an off-white solid (166 mg, 0.71 mmol, 85 %).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.37 (d, *J* = 2.5 Hz, 1H), 8.64 (dd, *J* = 8.5, 2.3 Hz, 1H), 8.42 (d, *J* = 8.5 Hz, 1H), 8.16 (app. s, 1H), 3.64 (app. q, *J* = 6.6 Hz, 2H), 2.33 (td, *J* = 6.9, 2.6 Hz, 2H), 2.02 (t, *J* = 2.6 Hz, 1H), 1.90 (app. p, *J* = 6.9 Hz, 2H). **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) 162.4, 154.2, 145.7, 143.9, 132.9, 123.0, 83.2, 69.6, 39.0, 28.2, 16.3. **HMRS-ESI** (*m/z*): found [M+H]⁺ 234.0868, C₁₁H₁₂N₃O₃ requires 234.0879.

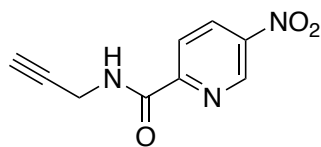
N-(5-nitropyridin-3-yl)hex-5-ynamide, 1b



Synthesised according to General Procedure K from 3-amino-5-nitropyridine (139 mg, 1.00 mmol) and 5-hexynoic acid (0.11 ml, 1.00 mmol) to afford the product as a pale orange solid (96 mg, 0.41 mmol, 41 %).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.16 (d, *J* = 2.3 Hz, 1H), 9.02 (app. t, *J* = 2.5 Hz, 1H), 8.90 (d, *J* = 2.3 Hz, 1H), 7.68 (s, 1H), 2.63 (t, *J* = 7.2 Hz, 2H), 2.36 (td, *J* = 6.7, 2.7 Hz, 2H), 2.04 (t, *J* = 2.7 Hz, 1H), 1.99 (app. p, *J* = 7.0 Hz, 2H). **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) 171.9, 145.1, 143.2, 137.3, 136.9, 123.2, 83.2, 70.0, 35.9, 23.57, 17.9. **HMRS-ESI** (*m/z*): found [M+H]⁺ 234.0876, C₁₁H₁₂N₃O₃ requires 234.0879.

5-nitro-N-(prop-2-yn-1-yl)picolinamide



Synthesised according to General Procedure K from 5-nitropyridine-2-carboxylic acid (141 mg, 0.84 mmol) and propargylamine (54 μ l, 0.84 mmol) to afford the product as a pale yellow solid (124 mg, 0.60 mmol, 72 %).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) 9.39 (d, $J = 2.5$ Hz, 1H), 8.65 (dd, $J = 8.6, 2.5$ Hz, 1H), 8.42 (d, $J = 8.6$ Hz, 1H), 8.15 (app. s, 1H), 4.30 (dd, $J = 5.6, 2.6$ Hz, 2H), 2.31 (t, $J = 2.6$ Hz, 1H). **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ (ppm) 162.0, 153.6, 145.9, 144.0, 132.9, 123.2, 78.8, 72.3, 29.7. **HMRS-ESI** (m/z): found $[\text{M}+\text{H}]^+$ 206.0557, $\text{C}_9\text{H}_8\text{N}_3\text{O}_3$ requires 206.0566.

Enzymatic synthesis of N⁶mdA-containing dsDNA

dsDNA was synthesised enzymatically by primer elongation with Vent® DNA Polymerase (NEB) using a non-methylated template (sequences see below), the according reverse primer (sequences see below) and either a set of canonical dNTPs (to synthesise non-methylated dsDNA) or a mixture dCTP, dGTP, N⁶mdATP and dTTP (to synthesise dsDNA with N⁶mdA at the indicated positions).

template 1

AACGGAAGCAGAACAGAACGAAGCAAGACGAGCAACACGAACAGAACACGAAACGA**T**GCAAGAGAGCAAG
CAAGCAACG**T**TCGTTGCTGTTTCGCTGTTG

template 2

AACGAAGCAGAACGCAGAAGAAGCAAGACGACCAACACG**T**ACGGAACACGAAACGAAGCA**T**GCGCCGAAG
CAAGCCACG**T**TCGGTTGCTGTTCTGTTTCG

reverse primer 1

CAACAGCGAACAGCAACGAA

reverse primer 2

CGAACAGAACAGCAACCGAA

5-10 parallel reactions were run with following optimised concentrations:

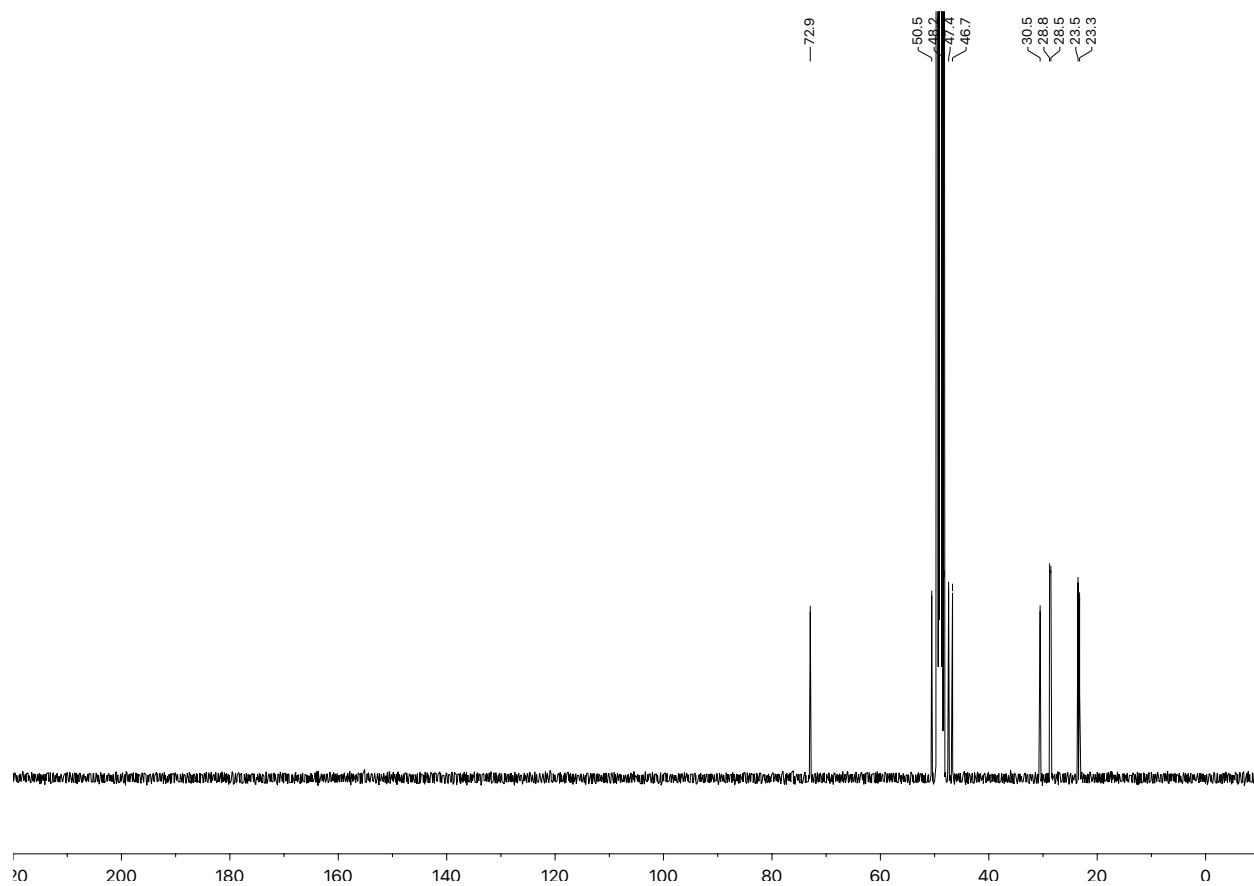
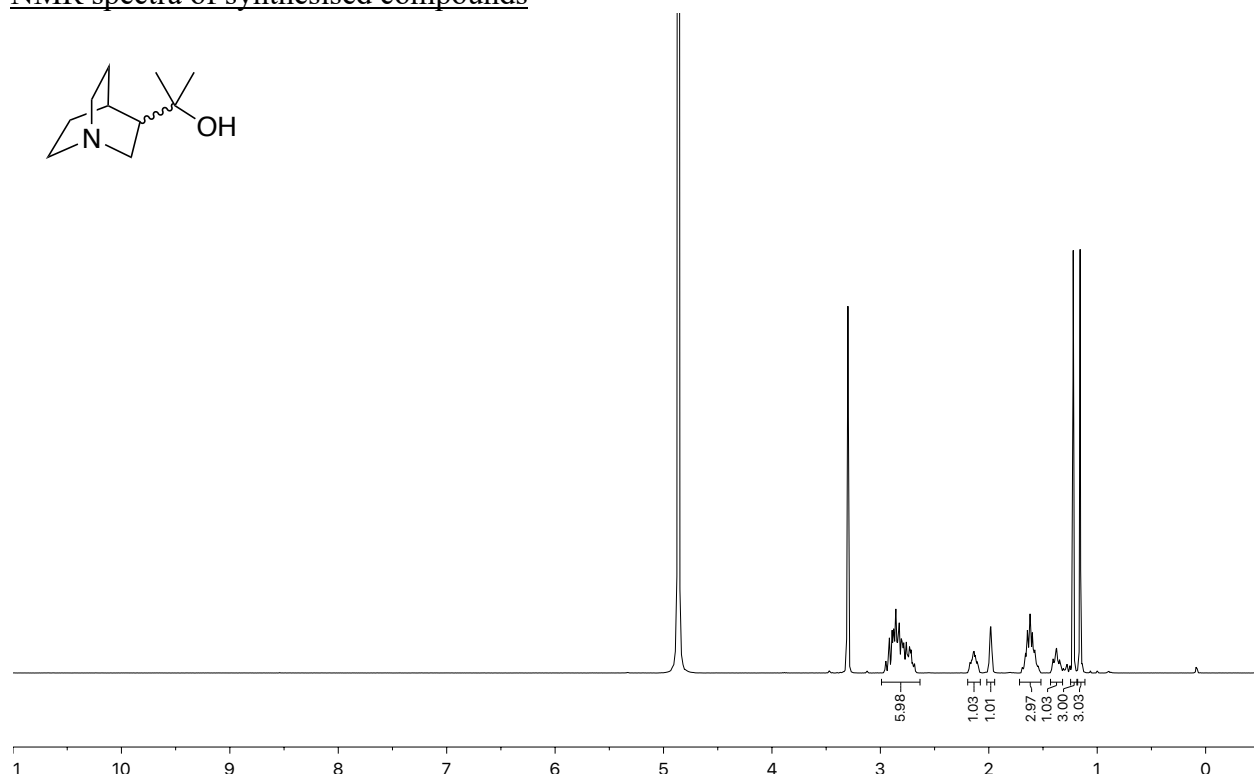
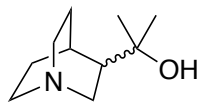
reagent	final c/amount
template	0.5 ug
primer	10 µM
Vent® DNA Polymerase (NEB)	2 U
ThermoPol® Reaction buffer (NEB)	1X
dATP or N ⁶ m-dATP	2 mM
dCTP	2 mM
dGTP	2 mM
dTTP	2 mM
H ₂ O	for a final volume of 20 µl

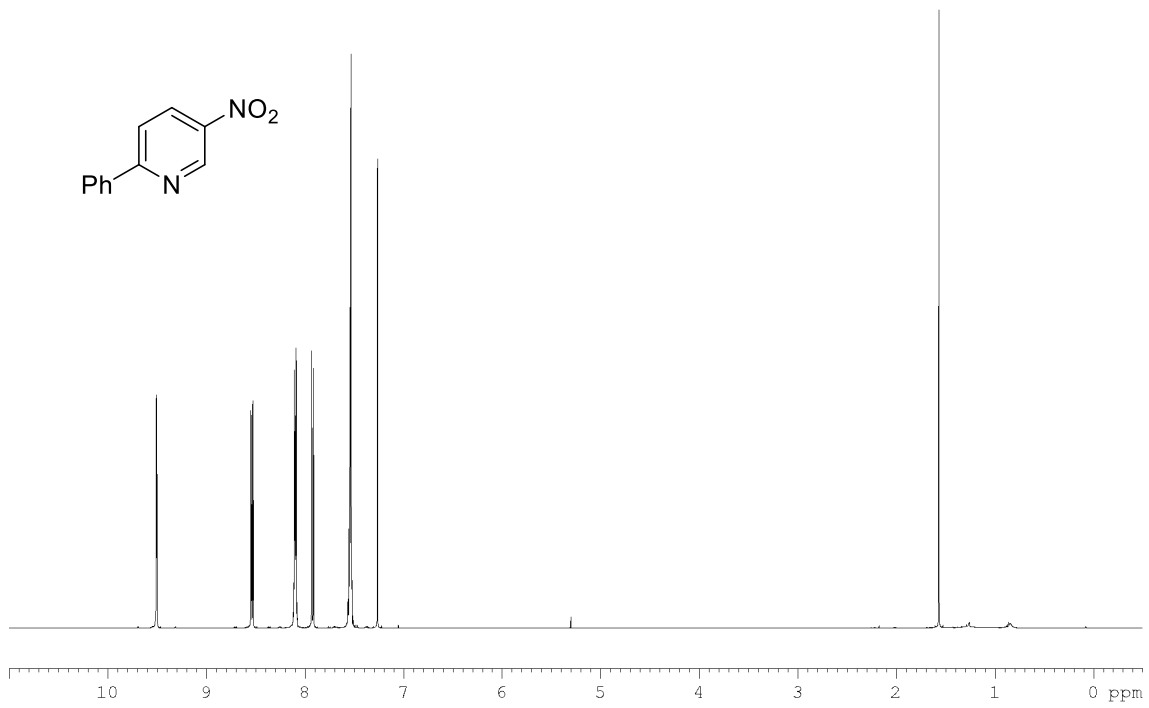
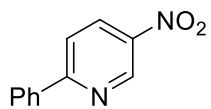
The reactions were run using the following optimised temperature cycle:

Step	Time	Temperature
melting/denaturing	30 sec	95 °C
primer annealing	1 min	60 °C
polymerisation	10 min	72 °C
storage	inf.	4 °C

Upon reaction, the parallel PCR reactions were combined and the PCR products purified with the GeneJet PCR purification kit (Thermo Scientific) according to the manufacturer's protocol (without the use of *i*PrOH). The products were analysed by automated gel electrophoresis (Tapestation) and LCMS to confirm the identity of the synthesised strands.

NMR spectra of synthesised compounds





1.000

1.059

2.166

1.102

3.195

162.64

145.42

143.03

137.22

132.09

131.04

129.28

127.84

120.21

