Selective chemical functionalization at N⁶mdA residues in DNA

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Abstract

Selective chemistry that modifies the structure of DNA and RNA is essential to understanding the role of epigenetic modifications. We report a visible-light-activated photocatalytic process that introduces a covalent modification at a C(sp³)–H bond in the methyl group of N6-methyl-adenosine—an epigenetic modification of emerging importance. A carefully orchestrated reaction combines reduction of a nitropyridine to form a nitrosopyridine spin-trapping reagent and an exquisitely selective tertiary amine-mediated hydrogen-atom abstraction at the N6-methyl group to form an α -amino radical. Cross-coupling of the putative α -amino radical with nitrosopyridine leads to a stable conjugate, installing a label at N6-methyl-adenosine. We show that N6-methyl-adenosine-containing oligonucleotides can be enriched from complex mixtures, paving the way for applications to identify this modification in genomic DNA and RNA.

Nucleic acids displays several types of C(sp³)–H bonds within its canonical nucleotides, each with subtly different intrinsic reactivities that are influenced by steric, inductive and conjugative effects imparted by the proximal chemical environment. Beyond the core genetic information stored the four-letter nucleotide sequences of A, C, G and T(U), nucleic acids contains a second layer of molecular programming in the form of reversible chemical modifications to its nucleobases – the, so called, epigenetic code – which introduces further diversity to the catalogue of C(sp³)–H bonds present in these biomacromolecules. Methylation at specific positions of the nucleobases provides the most common means by which genomic DNA is reversibly marked (*1,2*). Bacteria can methylate A and C in their own genome (*3*), while in eukaryotes, DNA methylation was thought to only occur at C (*2*), which has been linked to gene regulation. Importantly, the discovery of novel chemical methods for the selective modification of 5-methylcytosine (5mC) and its derivatives in DNA have been unequivocally responsible for a better understanding of its function (*4*). Recently, N6-methyl deoxyadenosine (N⁶mdA, Figure 1A) has been reported (somewhat controversially) in various eukaryote genomes (*5-16*) and comprehension of its biological roles remains nascent (*5,10*). In contrast, the presence of N6-methylation of adenine (m⁶A) in the RNA world is well-established and has been implicated in a wide range of cellular processes (*17*). Even though methyl groups within N–methylamines are not traditionally reactive, the exclusivity of this motif might underpin a site-selective chemical approach with which to covalently modify and manipulate N6-methyl-adenosine; there is currently no method to directly modify nucleic acids at N6-methyl-adenosine and a selective chemical transformation must target the C(sp³)–H bonds of the N–methylamine motif amidst thousands of similarly reactive entities.

We were inspired by the cellular processing of methylation state in oligonucleotide sequences, wherein a class of dioxygenases, which include ALKBH1, have been proposed to demethylate N⁶mdA in mammalian DNA (*9,11*) through the action of electrophilic enzyme-bound Fe(IV)=O intermediates (Figure 1b) (*18*). By analogy with the corresponding demethylation in RNA (*19*), the C–H bond of the N6–methyl group would project

towards the promiscuous Fe-bound oxygen-centred radical facilitating selective hydrogen atom abstraction (HAA), which is followed by an oxygen-rebound process with the resulting Fe(III)–OH species *en route* to formation of N⁶-hydroxymethyl-adenosine and spontaneous expulsion of formaldehyde. We questioned whether a synthetic process could generate a discrete α -amino radical intermediate on the N6-methyl group of N⁶mdA, mimicking the enzymatic demethylation pathway, but also intercept the incipient radical with a modular reagent, installing a covalently-bound label directly onto N⁶mdA-containing DNA sequences (Figure 1c).

Figure 1. Evolution of a strategy for the chemical modification of N⁶mdA in DNA. a. Canonical DNA bases and N⁶mdA. b. Proposed mechanism for biochemical demethylation of N⁶mdA via Fe-dependent enzyme-controlled hydrogen atom abstraction and oxygen-rebound. c. Plan for covalent modification at N⁶mdA via trapping of an 'on-DNA' α -amino radical intermediate.

The C–H bonds in N⁶mdA's methyl group have fairly high bond dissociation energies (BDE, ~92-94 kcal/mol)(20). A selective reagent will need to target a strong C–H bond that is present at extremely low effective concentration (reported N⁶mdA levels in eukaryotes have been reported from 5ppm to 0.05% for N⁶mdA with respect to A) (9,11,12), amongst a plethora of similar strength or weaker C–H bonds: for example, deoxyribose

units contain many different C–H bonds, each with similar BDE's (21); and the methyl group in thymidine (and 5mC) displays activated C–H bonds with lower BDE (89-90 kcal/mol) (22). Moreover, such a N⁶mdA functionalization strategy also necessitates a method to productively intercept the 'on-DNA' α -amino-radical to fashion a stable covalent linkage to the oligonucleotide. The challenges associated with addressing these problems are multifaceted: firstly, use of a proximity-driven rebound mechanism thought to facilitate enzymatic demethylation is unlikely to be feasible in a synthetic scenario and so the coupling step will need to be fast in order to accommodate the likely short lifetime of the DNA-derived α -amino-radical; and secondly, the HAA and covalent functionalization steps need to operate in concert while minimizing deleterious and non-selective reactivity. These difficulties place additional constraints on potential chemical solutions that must already operate at low concentrations, in aqueous solutions and avoiding acidic or oxidative conditions, which might damage the nucleic acid architecture.

The C–H bonds in the methyl group of N⁶mdA will be partially polarized as a result of their interaction with the lone pair on the N6-atom and display 'hydridic' character (23,24) (Figure 2A). In contrast, the C–H bonds of in the methyl group of thymine, although weaker, are relatively neutral as a result of being adjacent to the less polarizing pyrimidine heterocycle. This subtle electronic effect may provide a sufficiently distinct reactivity profile to enable a kinetically-controlled polarity match between an electrophilic hydrogen atom abstracting agent and the more hydridic C–H bonds in the N6-methylamine motif in N⁶mdA. The resulting C–H bond cleavage would lead to formation of an α -amino radical on the modified nucleotide. The intercepting reagent must react quickly with the incipient N⁶mdA-derived α -amino radical and form an open shell species more stable than its precursor. We reasoned that deployment of a spin trapping reagent could provide a potential solution to this challenge. Spin trapping reagents (STRs) are highly reactive molecules, frequently used in excess quantities to capture radicals in the form of persistent radical products, which can enable the identification of short-lived

species in complex systems (25). Among commonly used STRs, nitrosoarenes are particularly suitable for the interception of nucleophilic carbon-centred radicals, the properties of which should be inherent to a N⁶mdA-derived α -amino radical. Nitrosoarene-derived STRs are, however, highly electrophilic and often display promiscuous non-radical reactivity with nucleophiles, can undergo facile dimerization and readily decompose to non-productive products. To compound these problems, a nitrosoarene must also be compatible with the HAA step, itself a radical reaction, without displaying deleterious reactivity. We questioned whether these problems might be circumvented if a process could be designed wherein the STR was generated *in situ*, consequentially linked to the chemistry required to facilitate the HAA step, thereby closely linking the proximity of this reactive species to the incipient N⁶mdA-derived radical. Accordingly, we hypothesized that a mild method to reduce a water-soluble nitroarene might be leveraged alongside the HAA step for the *in situ* generation of a nitrosoarene STR and lead to a selective functionalization process (Figure 2B).

Our design plan for a C(sp³)–H functionalization of N⁶mdA focused on the visible light-mediated reduction of 3-nitropyridine **1a**, a water soluble oxidant that could serve as a precursor for the nitrosoarene STR (Figure 2C). We speculated that Ru(II)(phen)₃Cl₂ could function as a photocatalyst because it displays adequate aqueous solubility and the oxidative quenching cycle of its triplet excited-state ($E[\text{Ru(II)}^*/\text{Ru(III)}] = -0.87 \text{ V}$) (26), accessed by visible-light irradiation, is well matched to the reduction potential of the nitropyridine ($E^{\text{red}} = -0.44 \text{ V}$ vs SCE estimated based on the value for 4-nitropyridine) (27); nitropyridine-derived radical anion int **I** would be produced alongside [Ru(III)(phen)₃]. Int **I** could undergo hydrogen atom transfer with the bulk solvent (or perhaps N⁶mdA) to int **II** and eliminate water to form the STR, 3-nitrosopyridine **2a** (28). Crucially, the concentration of the 3-nitrosopyridine (**2a**) would be controlled by the photochemical activity of the catalyst, avoiding the presence of potentially deleterious super-stoichiometric levels of STR. Concurrent with the formation of the STR, the [Ru(III)(phen)₃] intermediate ($E[\text{Ru(III)}/\text{Ru(II)}] = +1.26 \text{ V}$) (29) would be reduced by

quinuclidine **3** ($E^{\text{ox}} = +1.10$ V vs SCE) (30), reforming the photocatalyst to close the cycle and, importantly, generate the quinuclidine radical cation (int **III**), a powerful electrophilic hydrogen atom abstractor. Protonated quinuclidine has a BDE of 101 kcal/mol (30) meaning that its radical cation could be sufficiently reactive to remove a hydrogen atom from the N⁶mdA-methyl group; MacMillan has shown that quinuclidine radical cation displays polarity-matched reactivity for strong electron-rich C–H bonds under mild reaction conditions via Ir-catalyzed photoredox-mediated single electron oxidation (24,29) Therefore, selective HAA from the methyl group in N⁶mdA by int **III** will generate the desired α -amino radical (int **IV**). Cross coupling of the 3-nitrosopyridine STR **2a** with α -amino radical int **III** would then generate a nitroxide persistent radical (int **V**) (31-34), which can ultimately undergo well-established oxidative decay to fashion a covalent modification at the N6-position of the nucleobase in the form of a N-hydroxyformamidine linkage **4** (35).

A — challenge 2: selective hydrogen atom abstraction at N⁶mdA —

B — challenge 1: in situ generation of spin trapping reagents (STRs) —

C — Design plan: N⁶mdA functionalization strategy —

Figure 2. Design plan for a N⁶mdA-selective functionalization process. **a.** Nitrosoarene-derived STRs; can a stable precursor, such as a nitroarene, be used for *in situ* generation of reactive spin trapping reagents? **b.** Selective HAA at N⁶mdA. **c.** Design plan for photoredox-facilitated covalent modification of N⁶mdA based on the merger of selective HAA and spin trapping via *in situ* generation of nitrosoarenes.

Our initial studies focussed on establishing a cross coupling protocol on a representative oligonucleotide **5** (CTTGACAG[N⁶mdA]CTAG, Figure 3A). Following an extensive assessment of the reaction parameters, the exploratory experiments revealed that irradiation of a solution of **5a**, 3-nitropyridine **1a**, quinuclidine **3** and [Ru(phen)₃]Cl₂ with a 60 W CFL bulb for just 10 minutes at room temperature, led to the formation of *N*-hydroxyformamidine-N⁶mdA oligonucleotide conjugate **6** with 16% conversion to product, as determined by LC-MS analysis (Figure 3a) (36). A 30% conversion to the de-methylated oligonucleotide **7** was also observed. It is important to stress that both conjugate **6** and demethylated oligonucleotide **7** arise from the same putative α -amino radical, int-III, which reflects a 46% conversion of the N6-methyl group via the new hydrogen atom abstraction process and is surprisingly high given the highly complex molecular framework upon which this transformation is affected. We believe that the constant oxidative quenching of triplet excited state of the metallophotocatalyst by 3-nitropyridine and high concentration of quinuclidine prevents oxidative damage of DNA, especially at G nucleobases. This is reflected in the observation that the transformation using the Ru(phen)₃Cl₂ as catalyst produces a cleaner reaction profile compared to the use of other, more oxidizing photocatalysts, [Ru(II)(bpz)₃](PF₆)₂ (Figure S18, supplementary information). The oligonucleotide conjugate (**6**) has a half-life of approximately 12 hours at room temperature in neutral or basic solutions (pH = 7-11).

Figure 3. A visible light-mediated photoredox strategy for covalent functionalization of N⁶mdA. **a.** Initial exploration of HAA at N⁶mdA: oligonucleotide conjugation via HAA and spin trapping via an oxidative photocatalytic quenching cycle with [Ru(II)(phen)₃]Cl₂. **B.** The use of modular nitropyridine probes in oligonucleotide functionalization and subsequent elaboration by Huisgen cycloaddition. **C.** Selectivity parameters in the oligonucleotide functionalization are defined as 'HAA selectivity' (reflecting the position of C–H bond cleavage) and 'Probe selectivity' (reflecting the selectivity of reaction via nitrosopyridine vs nitropyridine). **D.** Scope of N⁶mdA functionalization using standard conditions detailed in **A** and **B**. For the reaction of **5c**, Ru(bpz)₃(PF₆)₂ was used as catalyst.

We next sought to incorporate a latently reactive functionality capable of downstream elaboration to tailored nucleic acid fragments. A design-augmentation process revealed an alkyne-containing, amide-linked nitropyridine **1b** could be coupled with **5a** upon treatment with the Ru(phen)₃Cl₂, quinuclidine and irradiation for 10 minutes (Figure 3B), forming the desired alkyne-containing oligonucleotide **8** (11% conversion to product, identified by HRMS). It is notable that **1b** is devoid of potentially competitive hydridic C–H bonds and the amide substituent does not seem to affect the oxidative reactivity of the 3-nitropyridine core. Exploiting the newly installed alkyne functionality, we found that a ‘click’ Huisgen-cycloaddition between **8** and PEG₃ biotin-derived azide **9** necessitated specific conditions for an effective reaction; a solution of copper sulfate and sodium ascorbate required the addition of quinuclidine (presumably to act as a ligand for the copper-catalyst) to facilitate cycloaddition to the biotin-conjugated oligonucleotide **10** with 92% conversion to product.

A series of control experiments showed that the photoredox coupling reaction on an oligonucleotide without a N⁶mdA residue (CTTGACAGACTAG, **7**) formed no N-hydroxyformamidine-containing products arising from the incorporation of a 3-nitrosopyridine unit, indicating, as expected, that hydrogen atom abstraction does not take place in the oligonucleotide unless the N6-methylation is present. It is remarkable that the hydrogen atom abstraction step is so exquisitely selective for the N6-methyl group in spite of the vast number of similar C–H bonds in oligonucleotides (termed ‘HAA selectivity’, Figure 3C). We were, however, able to detect trace levels of oligonucleotides that had a mass ion reflecting the inclusion of an intact 3-nitropyridine (16 mass units higher than N-hydroxyformamidine-derived oligonucleotide **8**). Although we were not able to elucidate the structure of this trace-level modification, a series of control experiments revealed that the addition of 3-nitropyridine was taking place at G residues (Figure S20-21, supplementary information). We were able to calculate that selectivity for the formation of the desired N⁶mdA-derived N-hydroxyformamidine linkage compared to the inclusion of 3-

nitropyridine at G was 50:1 for N⁶mdA per G nucleobase (termed ‘Probe selectivity’, Figure 3C), a ratio which is, again, quite remarkable given the proclivity of G nucleobases to undergo oxidative side reactions.

Figure 3D shows a preliminary scope of the N⁶mdA functionalization tactic, with the conversions to conjugates in line with those observed in the optimization studies. Not only did the reaction work on oligonucleotides in combination with nitropyridines **1a** and **1b** but it also converted longer and self-complimentary DNA sequences (42 & 49mers) to the desired products. Importantly, a reaction with an RNA oligonucleotide was successful converted to the corresponding conjugate although the yields were lower than for the DNA congeners; partial strand decomposition was observed in the RNA oligonucleotide, which will require further optimization of the reaction conditions. Nevertheless, the success of this methodology on RNA oligonucleotides has many potential applications in the emerging field of m⁶A-focussed epitranscriptomics (17).

The versatile biochemical properties inherent to the biotin motif provide a means to isolate the modified N⁶mdA-derived oligonucleotide from other nucleic acid fragments via a streptavidin-based pull-down procedure (37), which could enable us to enrich N⁶mdA-containing oligonucleotides in complex mixtures. Classical methods for substrate retrieval from streptavidin pull-down protocols involve relatively harsh reaction conditions, which are designed to denature the protein scaffold. However, our photoredox conjugation procedure installs the N-hydroxyformamidinium linkage, a more labile functional group, which we believed would permit the use of significantly milder, nucleophile-mediated, cleavage conditions in the retrieval of the labelled oligonucleotide. This is important because mass analysis of the photoredox reaction mixtures had suggested that the traces products arising from unselective functionalization at G do not contain an electrophilic N-hydroxyformamidinium linkage. Consequently, we speculated that these off-target products of functionalization at G could be retained on the streptavidin beads during cleavage, thereby enhancing the selectivity observed in the photoredox step and enrichment of the N⁶mdA-derived oligonucleotide. Guided by this hypothesis, we began the pull-down and

enrichment procedure by conducting the photo-conjugation with oligonucleotide **5a** and nitropyridine **1b** in the presence of a distinct, but importantly non-methylated oligonucleotide CGTACTAGACG **11**, as a means to test whether our method could be used to enrich N⁶mdA-containing oligonucleotides (Figure 5a). The N-hydroxyformamidine product **8** was formed with 10% conversion and observed by LC-MS alongside unreacted **5a**, the demethylated oligonucleotide **7**, the control oligonucleotide **11** and traces of the G-nitropyridine functionalized oligonucleotide (50:1 probe selectivity, N⁶mdA/G). Subsequent cycloaddition with biotin-azide **9** afforded the selective N⁶mdA biotin-conjugated oligonucleotide **10**. Treatment of the oligonucleotide mixture with streptavidin-coated magnetic beads allowed immobilisation of all the species containing biotin (specifically, **12** plus the trace levels of product arising from unselective reaction at G residues) and permitted the removal of unlabelled oligonucleotides (**7** and **11**) via successive washing procedures. Following this, we found that the electrophilic nature of the N-hydroxyformamide linkage made it susceptible to reaction with aqueous hydrazine and led to the release of an N⁶-(hydrazonomethyl)dA-containing oligonucleotide **13** with a small amount of **7** (arising from the hydrolysis of **13**) and trace quantities of the other oligonucleotides that had been indiscriminately retained by the streptavidin-coated beads. The recovery of both **13** and **7** provides direct evidence for the presence of N⁶mdA in the starting sequence and their ratio to all other oligonucleotides gives rise to an enrichment greater than 50:1. It is particularly important to note that the maximum theoretical enrichment value that can be obtained as a result of the observed photoredox probe selectivity is ~17:1, since oligonucleotide **13** contains 3 G residues (probe selectivity of 50:1 N⁶mdA per G residue). Therefore, the observed enrichment of >50:1 clearly demonstrates that the hydrazine cleavage procedure is selective for N-hydroxyformamidine linkage in the N⁶mdA-derived oligonucleotide conjugates versus products of reaction at G (that are presumably retained on the beads), leading to the observed enhanced enrichment.

Figure 4. A pull-down strategy for the enrichment of N⁶mdA-containing oligonucleotides. A. A pull down procedure involving photoredox functionalization with an alkyne-derived nitropyridine, Huisgen cycloaddition with a biotin-derived azide, immobilization on streptavidin coated magnetic beads, oligonucleotide separation by sequential washing and selective cleavage of N⁶mdA-derived oligonucleotides delivers an enrichment of >50:1. **B.** Pull down experiments using 99nt ssDNA and 99bp dsDNA in the presence and absence of salmon sperm (SS) DNA demonstrates enrichment in complex mixtures of DNA sequences; blue dot indicates position of N⁶mdA. dsDNA displaying the methyl group (red) of the N⁶mdA nucleobase (blue) in the major groove.

To simulate the complex matrix of a cellular DNA sample, where the concentration of N⁶mdA with respect to dA will be very low, we combined longer single-stranded (ss) DNA fragments (99 nucleotides) with a 10-fold

excess of salmon sperm DNA, to create complex DNA mixtures with N⁶mdA/dA ratios of 1:383 (0.26%). Applying the photo-conjugation and pull-down procedure to these samples and now using quantitative PCR (qPCR) to analyse the enriched fractions and determine the amplifiable amount of both initially methylated and unmethylated DNA sequences after the pull-down, we found that the N⁶mdA-containing ssDNA fragment (yellow strand, Figure 5b, experiment i) was enriched to a level of approximately 10:1. Importantly, a parallel experiment (ii) using the same nucleic acid sequences but having the N⁶mdA residue in the other sequence (grey) showed a similar level of enrichment (11:1). These results clearly demonstrate that N⁶mdA is required for the enrichment, the observed enrichment is not dependent on the oligonucleotide sequence and that the protocol is functional in complex DNA mixtures.

In double stranded DNA, the methyl group of N⁶mdA is thought to project into the major groove of the double helix, providing an additional challenge for the photoredox functionalization of complex nucleic acid samples due to potentially adverse steric and electronic effects that arise from the local chemical environment. Despite this congestion, we found that on applying the sequential photoredox conjugation, click reaction and pull-down procedure to a mixture of N⁶mdA-containing 99 base pair dsDNA (blue double strand, iii) and a non-methylated double stranded fragment (green double strand) resulted in an enrichment of 4:1. A parallel experiment (iv) using the same nucleic acid sequences but having two N⁶mdA residues in the second template showed that the N⁶mdA-containing dsDNA was recovered with an enrichment of greater than 9:1, indicating a positive cumulative effect. Furthermore, when these dsDNA fragments were combined with salmon sperm DNA (N⁶mdA/dA ratio is 1:3433, 0.03%), the N⁶mdA-containing oligonucleotide was again recovered with an enrichment of almost 4:1 (experiment v). The corresponding experiment (vi) using the same nucleic acid sequences but with two N⁶mdA residues in the second template (N⁶mdA/dA ratio is 1:1717, 0.06%) showed an increased enrichment of greater than 8:1 for the N⁶mdA-derived ds-oligonucleotide, again highlighting that the presence of additional N⁶mdA residues enhances the output. Taken together, this set of experiments demonstrates the applicability of the

developed chemistry on longer ssDNA, dsDNA, as well as in complex samples with excess DNA, providing a proof of concept for the enrichment of N⁶mdA-DNA strands and showcasing the potential for future application with this underexplored methylated nucleotide.

While the cell's biochemical machinery is capable of regulating the methylation state of A in nucleic acids, we have developed a selective chemical transformation that generates and intercepts an 'on-DNA' α -amino radical to form a stable covalent modification at N⁶mdA residues. Orchestrated by a visible light-activated photoredox catalyst, a polarity-matched hydrogen atom abstraction step at the N6-methyl group of N⁶mdA generates an α -amino radical and dovetails with a distinct, in situ, reaction to form a nitrosopyridine spin trapping reagent, which together lead to radical cross coupling process that introduces a modular functional handle into oligonucleotide sequences. This strategy is underpinned by a previously unknown transformation founded on a mechanistically unique and remarkably selective photoredox cross coupling reaction, which targets a traditionally unreactive and scarce motif amidst the complex scaffold of nucleic acids.

To set this work in context, the evolution of this synthetic method towards a basic technology upon which a chemical method for locating N⁶mdA in genomic DNA can be founded, will require a number of further challenges to be addressed, despite the remarkable selectivity's observed in this functionalization process. Firstly, while the photocatalytic hydrogen atom abstraction process proceeds with competent efficiency (46% conversion—combination of conjugation and demethylation—with respect to the starting oligonucleotide), the spin trapping of the N⁶mdA-derived oligonucleotide radical requires improvement in order to increase the yield of the desired conjugation product. Secondly, the already high (50:1) probe selectivity for reaction at N⁶mdA per G residue could be further increased because the effective discrimination of the process becomes diminished as the length of the oligonucleotide increases (due to the increased concentration of G residues in complex DNA).

Finally, further investigation of the unexplored reactivity of N-hydroxyformamidines should lead to improved chemoselectivity in the release step of the pull-down procedure and higher final enrichment. Together, the next phase of these studies will focus on the development of a detection protocol for N⁶mdA-containing oligonucleotides that already returns an enrichment of 4:1 for a single N⁶-methylation in a 99 base pair double stranded fragment that is part of a complex DNA matrix. Not only might this technology provide an unequivocal answer to the controversy surrounding the presence of N⁶mdA in genomic mammalian DNA but could also lead to sequencing methods that will further unravel the role of this epigenetic modification and should also be amenable to targeting methylated nucleobases in the many forms of RNA that regulate cellular function (17, 38).

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Supplementary Materials:

Materials and Methods

Figures S1-S31

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Supplementary Materials for

Selective chemical functionalization at N⁶mdA residues in DNA

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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 (125 Å, 2.5 μm , 2.1x50mm) with TMS endcapping or an XBridge Oligonucleotide BEH C18 column (130 Å, 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 (125 Å, 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 *N*⁶mdA 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 H_2O , 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 $[Ru(bpz)_3](PF_6)_2$ (2 mg in 1 mL of 20% MeCN in milliQ H_2O , 2.31 mM, 12.5 μ L) or $Ru(Phen)_3Cl_2$ (1.5 mg in 1 mL of 20% MeCN in milliQ H_2O , 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 H_2O), azide-PEG₃-biotin conjugate (20 mM, 5 μ L), sodium ascorbate (40 mM, 5 μ L) and $CuSO_4$ (2 mM, 5 μ L). Final concentrations: quinuclidine 1 M, azide-PEG₃-biotin conjugate: 2 mM, sodium ascorbate: 4 mM, $CuSO_4$ 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 H_2O (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 $\text{Ru(phen)}_3\text{Cl}_2$ 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 $\text{Ru(phen)}_3\text{Cl}_2$ 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 $\text{Ru(phen)}_3\text{Cl}_2$.

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 photosensitizer [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 $[Ru(bpz)_3](PF_6)_2$ and reduced concentrations of quinuclidine

To understand if excited $[Ru(bpz)_3](PF_6)_2$ 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-hydroxyformamidine 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-hydroxyformamidine conjugate [N⁶mdA-P] **6** in 14% yield (Fig. S6), along with quinuclidine product [N⁶mdA-Q] (3% yield) and demethylation product [dA] (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-hydroxyformamidine 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.

<div style="display: flex; align-items: center; justify-content: space-around;"> <div style="text-align: center;"> <p>CTTGACAG[N⁶mdA]CTAG 5, (100 μM) [M-3H]³⁺ = 1323</p> </div> <div style="text-align: center;"> <p>N⁶mdA functionalization</p> <p>2 M quinucidine 3 3-nitropyridine der. 1.1 M [Ru(bpz)₃](PF₆)₂</p> <p>H₂O–MeCN (9:1), 15 W CFL 5-60 mins</p> </div> <div style="text-align: center;"> <p>CTTGACAG[N⁶mdA-PX]CTAG</p> </div> <div style="text-align: left;"> <p>+ CTTGACAG[dA]CTAG 7, [M-3H]³⁺ = 1318</p> <p>+ CTTGACAG[N⁶mdA-Q]CTAG [M-4H]³⁺ = 1359</p> </div> </div>					
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 **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-(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 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.

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

Fig. S14. 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]** ODN **9** peak. * denotes non-ODN signals probably arising from 5-nitro-*N*-(pent-4-yn-1-yl)picolinamide-derived species that were not completely removed by size-exclusion filtration.

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 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.

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 N⁶mdA 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 N⁶mdA 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-hydroxyformamidinium 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-hydroxyformamidinium 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, $m/z = 1399.92$, 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-hydroxyformamidinium 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 (ATCTACACTGACT). 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-hydroxyformamidinium linkage.

Collection of evidences for the characterisation of N-hydroxyformamidine 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-hydroxyformamidine 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-hydroxyformamidine structural linkage

The N-formamidine 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-hydroxyformamidine 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**.

Fig. S21. Hydrolysis studies of the formed ‘on-DNA’ N-hydroxyformamidinium structure to confirm its identity.

The three products **14**, **15**, and **16** were characterized by HRMS (Fig. S22 and S23).

Fig. S22. Extracted ion chromatogram of N⁶fdA product **14** (red colour, m-3/3 = 1327.56, 100 ppm range, magnification factor 10) before and after the acidic hydrolysis.

Fig. S23. Extracted ion chromatograms of 3-N-hydroxypyridine derivative **15** (blue colour, m+H = 220.1081, 10 ppm range, magnification factor 100) and 3-N-formyl-N-hydroxypyridine derivative **16** (red colour, m+H = 248.1030, 10 ppm range, magnification factor 100) after the acidic hydrolysis.

Importantly, a control experiment where oligonucleotide **7** was subjected to the same procedure (photoredox and hydrolysis) did not result in formation of any of the hydrolysis products according to scans of calculated exact masses with error ranges of up to 100 ppm. **Together, these results are consistent with the presence of a N-hydroxyformamidinium linkage in the photoredox N⁶mdA product.**

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

Fig. S24. Reaction of the formed ‘on-DNA’ N-hydroxyformamidine with hydrazine to form the N-NH₂ formamidine 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 formamidine 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-hydroxyformamidine moiety.**

Another indication of the presence of the N-hydroxyformamidine 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-hydroxyformamidine 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-hydroxyformamidine moiety (**8** and **10**). **The formation of stable complexes of the generated N-hydroxyformamidines 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).**

DNA 41mer **5c** sequence:

ATCGTACTAG [**N⁶mdA**] CTCAACGTCTTAGTGGGTGGTCGGTTCGCG

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**. THTPA: 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 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.

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 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, washing off unmethylated strands, then cleavage and retrieval of the initially methylated sequence, as depicted in Fig. S29. Importantly, selective nucleophile-mediated cleavage of the N-hydroxyformamidinium using an aqueous hydrazine solution was expected to afford high enrichment factors.

Fig. S29. Detailed procedure of the developed N⁶mdA-specific enrichment procedure with different checkpoints for the LCMS-based analysis of the outcome (see. Fig. S30).

The enrichment experiment was monitored by LCMS (Fig. S30)

Fig. S30. LCMS traces at different checkpoints of the performed chemical pull-down (Fig. S29) of the N⁶mdA-containing ODN [N⁶mdA] in the presence of the unmethylated ODN. 1) After treatment with streptavidin-coated magnetic beads, LCMS of the supernatant indicates full removal of [N⁶mdA-PX-B] from the mixture. 2) LCMS of the first washing mixture shows the three non-biotinylated species [N⁶mdA], [dA] and [dA]-ctrl. 3) after first incubation with 0.1 M NaOH, residual [dA]-ctrl and [dA] were detected. 4) Elution with 10% hydrazine in H₂O releases the selective cleavage product [N⁶mdA-NH-NH₂] with demethylated [dA] species and only traces of the other sequence [dA]-ctrl.

LCMS analysis of the different fractions clearly showed that an N⁶mdA-specific enrichment of >50:1 (for a) in Fig. S29/S30) and >30:1 (for b) in Fig. S29/S30) was achieved. This confirms that the developed chemistry can be used for N⁶mdA-specific enrichment of DNA strands

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 and quantified both sequences by qPCR after enrichment (Fig. S31). We also added excess salmon sperm DNA (SS-DNA) in an additional set of experiments to show that the presence of additional DNA does not interfere with the N⁶mdA-selective chemistry.

Fig. S31. Top: Scheme of the enrichment procedure of a synthetic adenine N⁶-methylated 99nt ssDN strand from a mixture with a different synthetic nonmethylated sequence. Bottom: outcome for the enrichment experiments (fold enrichment of amplifiable N⁶mdA-containing strand) with and without added salmon sperm DNA (SS-DNA) as determined by qPCR. Mean values of two independent experiments (where error bars present), error bars indicate standard deviation. Where SS-DNA was added, the calculated N⁶mdA/A ratio is 1:383 (0.26%).

Enrichment factors of 6:1 and 10:1 in the presence or absence of SS-DNA demonstrate that the developed chemistry also allows for an N⁶mdA-specific enrichment of 99nt DNA strands. Furthermore, excess background DNA does not interfere with the process.

Enrichment studies of 99bp dsDNA

To test application of the developed N⁶mdA-specific enrichment protocol on dsDNA, we applied it on a set of two 99bp dsDNA strands and quantified the outcome by qPCR (Fig. S32). Again, we added excess SS-DNA in an additional set of experiment to show that the presence of additional DNA does not interfere with the N⁶mdA-selective chemistry.

Fig. S32. Top: Scheme of the enrichment procedure of a synthetic adenine N⁶-methylated 99bp dsDNA strand from a mixture with a different synthetic nonmethylated sequence. Bottom: outcome for the enrichment experiments (fold enrichment of amplifiable N⁶mdA-containing dsDNA sequence) with and without added salmon sperm DNA (SS-DNA), as determined by qPCR. Mean values of two independent experiments (where error bars present), error bars indicate standard deviation. Where SS-DNA was added, the calculated N⁶mdA/A ratio is 1:3433 (0.03%) for dsDNA fragment (blue) containing one N⁶mdA and 1:1716.5 (0.06%) for dsDNA fragment (orange) containing two N⁶mdA.

Enrichment factors of 4:1 (for 99bp dsDNA containing one N⁶mdA residue) and of 8:1 resp. 9:1 (for 99bp dsDNA containing two N⁶mdA residues) confirmed applicability of the developed protocol on dsDNA, as well as a positive cumulative effect in the enrichment factors for higher N⁶mdA densities.

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 %).

^1H 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}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

AACGGAAGCAGAACGAAACGAAGACGAGCAACACGAACAGAACACGAAACGA**T**GCAAGAGAGCAAG
CAAGCAACGTTTCGTTGCTGTTTCGCTGTTG

template 2

AACGAAGCAGAACGCAGAAGAAGCAAGACGACCAACACG**T**ACGGAACACGAAACGAAGCA**T**GCGCCGAAG
CAAGCCACGTTTCGGTTGCTGTTCTGTTCTG

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