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Investigation of the Pathways of Excess Electron Transfer in DNA with Flavin-Donor and Oxetane-Acceptor Modified DNA Hairpins

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Abstract: Oxetane is a potential intermediate that is enzymatically formed during the repair of (6–4) DNA lesions by special repair enzymes (6–4 DNA photolyases). These enzymes use a reduced and deprotonated flavin to cleave the oxetane by single electron donation. Herein we report synthesis of DNA hairpin model compounds containing a flavin as the hairpin head and two different oxetanes in the stem structure of the hairpin. The data show that the electron moves through the duplex even over distances of 17 Å. Attempts to trap the moving electron with N_2O showed no reduction of the cleavage efficiency showing that the electron moves through the duplex and not through solution. The electron transfer is sequence dependent. The efficiency is reduced by a factor of 2 in GC rich DNA hairpins.

Keywords: DNA · DNA repair · electron transfer · flavin · photolyases

Introduction

Electron-transfer processes through the DNA duplex can occur over large distances.[1–7] Several groups have shown that a positive charge, generated in DNA after single-electron abstraction from the nucleoside deoxyguanosine, hops through the duplex by using either deoxyguanosines or deoxyadenosines as stepping stones.[8,9] Excess electrons in contrast hop through the duplex using the pyrimidine nucleosides deoxycytidine or deoxythymidine as stepping stones.[10,11] The sequence dependence of the long range hole transfer process was found to be strong. Best hopping efficiencies were determined for deoxyguanosine-rich sequences.[8,12,13] In contrast, the sequence dependence of the alternative excess electron-transfer process is less well understood. Recent measurements with electron donor and acceptor modified DNA duplexes showed, however, that the excess electron-hopping process is most efficient through deoxythymidine sequences and less efficient if deoxycytidines space the electron donors and acceptors in the duplex.[14–18]

One of the main remaining questions associated with the excess electron-transfer process is the exact electron-transfer pathway. In principle, the electron can either hop through the duplex or, alternatively, it can escape from the duplex and move along the double helix or through the solvent in form of a solvated electron as proposed by Harriman.[19]

Results and Discussion

In order to shine more light on the excess electron-transfer pathway in a DNA duplex we prepared the two series of flavin **1** bridged DNA hairpins (**2a–d** and **3a–d**) depicted in Figure 1. Hairpins have in contrast to normal DNA duplexes the advantage that they possess stable double helix structures and concentration independent melting points. The constructed DNA hairpins contain first of all flavin **1** as the cap. This unit injects, in its reduced and deprotonated state, after light excitation, electrons into the duplex.[20] The hairpins contain in the stem in addition one of two different oxetane units **2** and **3** as electron acceptors. Oxetanes have in general the advantage that they cleave very rapidly and irreversibly after single-electron reduction with rate constants between $10^{-7}\ s^{-1}$ and $10^{-8}\ s^{-1}$.[21–31] To use oxetanes as electron acceptor has in addition the appeal in that these compounds are believed to be intermediates in the repair of (6–4) DNA lesions by (6–4) photolyases. Here to, repair is assumed to involve single-electron donation followed by cleavage of the oxetane intermediate. In fact cleavage of oxetanes with various electron donors to simulate and investi-

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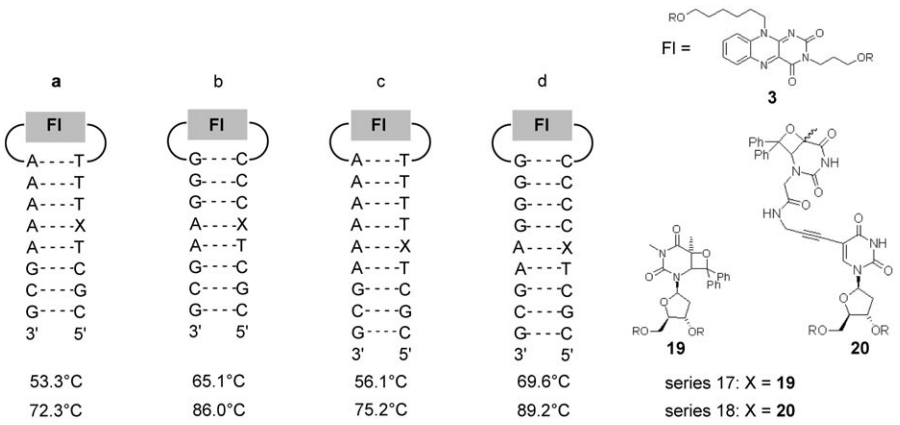


Figure 1. Depiction of the two series **2a–d** and **3a–d** of flavin (**1**) capped, oxetane containing oligonucleotide hairpins. Temperatures shown below the structures are the UV melting points. T_m measurement conditions: Hairpin concentration = 3 μM in buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4).

gate the process behind the repair reaction is of great actuality.[26,28,32] The hairpin series **2a–d** contains an oxetane, which is positioned close to the duplex. The assumed structure of the hairpins containing acceptor **2** is depicted as the result of a modeling study in Figure 2a. It is evident that the oxetane acceptor **2**, close to the duplex distorts the helix at the oxetane position.

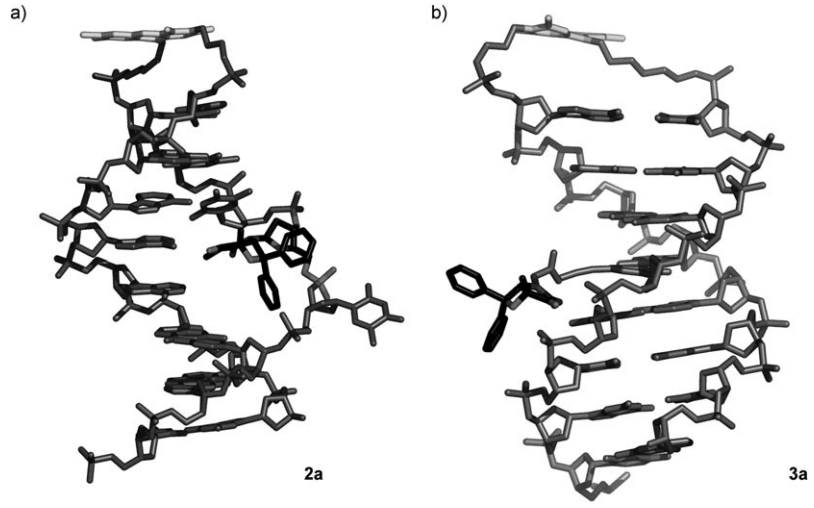


Figure 2. Result of modeling studies of the hairpins a) **2a** and b) **3a** (SYBYL 6.8 MMFF94, hairpin **2a** 8 ps MD in water, hairpin **3a** energy minimized structure).

The oxetane in series **3** is placed far outside the duplex structure as shown in Figure 2b. A semi-rigid propargylamide spacer places this electron acceptor at a distance of about 10 Å away from the nucleotide stack. In this hairpin series the duplex structure is rigid and closed. The oxetane acceptor points out of the major groove into the solution.

If the excess electron moves through the duplex using the bases as stepping stones, cleavage of the oxetane acceptor in series **2** should be fast. If however, the electron travels externally along the DNA duplex as a solvated electron, one would expect faster cleavage of the oxetane in series **3**. More importantly, in this scenario one should be able to disrupt the electron-transfer process upon addition of N_2O, which is a strong scavenger for solvated electrons.

In order to investigate again the sequence dependence of the electron movement along the DNA we also changed the nucleotide sequences in both hairpin series between the flavin cap and the oxetane acceptor from 3AT and 4AT (a and c) to 3GC and 4GC (b and d), respectively.

Synthesis: The flavin **1** *H*-phosphonate was prepared as previously described.[33] For the synthesis of the oxetane acceptor **2**, we first methylated 2-thiothymine **4** (Scheme 1) with methyl iodide and converted the resulting (2*S*)-methyl-3-methylthymine **5** into 3-methylthymine **6** using HCl at 80 °C.[34] Irradiation of 3-methylthymine **6** in the presence of benzophenone in acetonitrile in a quartz apparatus furnished in 44% yield the oxetane **7** as a pair of enantiomers. Subsequent glycosidation of **7** with α-1-chloro-3,5-di-*O*-toluoyl-1-deoxyribofuranoside gave the corresponding nucleoside **8** in 53% as a 1:1 mixture of the α,β-anomers. The diastereomerically pure β-anomer **8** [(5*S*)-configuration] was separated and the toluoyl protecting groups were cleaved to give compound **9**, which was finally converted into the phosphoramidite **2**. The crystal structure of compound **8** (Figure 3) confirmed the structure of the oxetane. Most interesting was the observation that only one diastereomer of the β-nucleoside **8** was obtained. We believe that the (5*S*)-configured oxetane (*S*)-**7** reacted selectively with the α-1-chloro-3,5-di-*O*-toluoyl-1-deoxyribofuranoside to give the corresponding β-anomer, while the (5*R*)-oxetane forms selectively the α-anomer. To our knowledge this is the first time that the stereochemical information present in the nucleoside determines the configuration of the anomeric center in the glycosidation reaction.

For the preparation of oxetane acceptor **3** we first alkylated thymine **11** with benzyl bromoacetate to give thymine

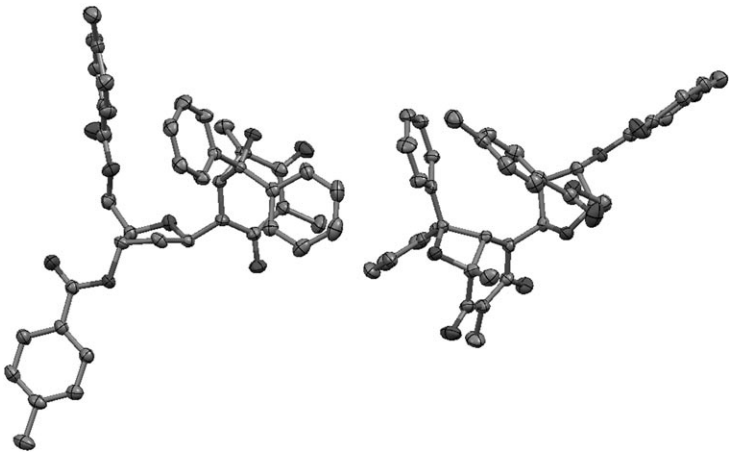
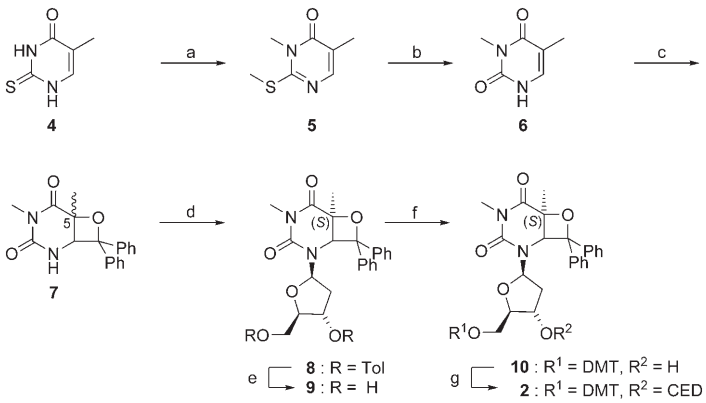


Figure 3. X-ray crystal structure (two representations) of compound **8** confirms formation of just one diastereomer of the β-anomer (for detailed information see Experimental Section).



Scheme 1. Synthesis of the oxetane electron acceptor **2**. a) MeI, DMF, RT, 18 h, 65%; b) 3 N HCl, 80 °C, 8 h, 93%; c) *hv*, benzophenone, MeCN, 3 h, 44%; d) α-1′-chloro-3′,5′-di-*O*-toluoyl-deoxyribofuranoside, NaH, MeCN, RT, 4 h, 53% α/β 1:1; e) K_2CO_3, MeOH, RT, 3 h, 84%; f) DMTCl, pyridine, RT, 4 h, 84%; g) β-cyanoethyltetraisopropylphosphordiamidite, diisopropylethylamine, THF, RT, 2 h, 67%.

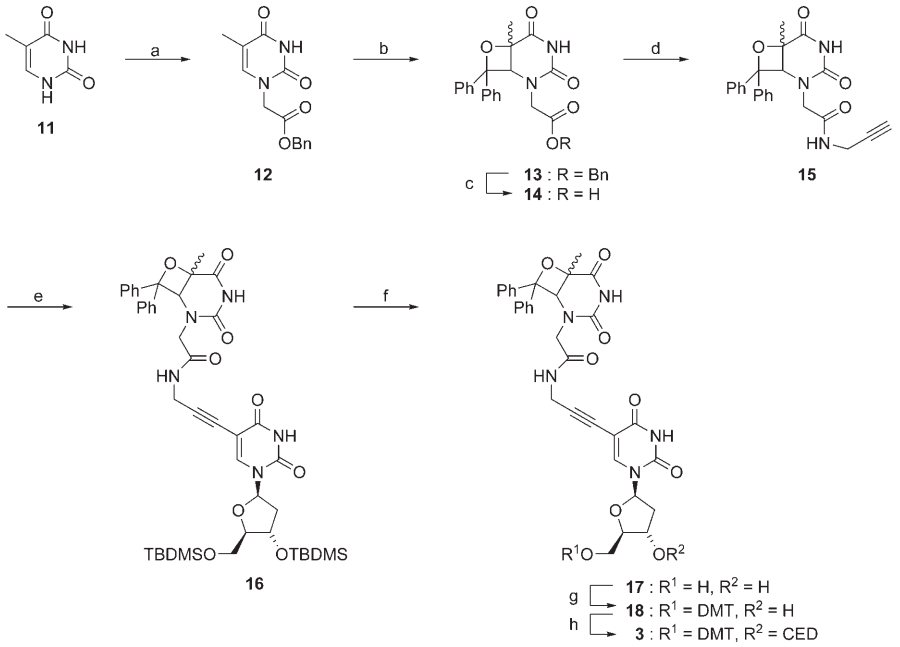
benzyl ester **12** (Scheme 2). We then irradiated compound **12** in the presence of benzophenone in a quartz apparatus to obtain the thymine-oxetane **13** as a racemic mixture.[25] Hydrogenolytic cleavage of the benzyl ester **13** over Pd/C in an H_2 atmosphere to carboxylic acid **14**, activation of the acid with TBTU/HOBt and reaction with propargylamine furnished the alkyne **15**. Sonogashira coupling of this oxetane–alkyne conjugate to the unprotected 5-iododeoxyuridine proved to be more difficult than anticipated and gave only low yields (28%). To improve the yield, we turned to a protected nucleoside such as the 3,5-bis-*tert*-butyldimethylsilyl-(TBDMS) protected 5-iododeoxyuridine. Sonogashira coupling with this compound furnished the oxetane–deoxyuridine conjugate **16** in a much better and much more reliable yield (66%) as a mixture of two diastereoisomers. Cleavage of the TBDMS groups and conversion of the unprotected nucleoside **17** into the DMT-protected phosphoramidite using standard procedures furnished the oxetane-acceptor building-block **3** ready for incorporation into DNA hairpins of series **3a–d** using solid-phase phosphoramidite chemistry.

Synthesis of the hairpins **2a–d** and **3a–d**, via phosphoramidite chemistry, using the building blocks **2** and **3** was performed on an Expedite DNA synthesizer with standard procedures. Highly base labile protected phosphoramidite building blocks were used. The DNA hairpins were after completion of synthesis cleaved from the solid support under concomitant cleavage of all protecting groups with 25% aq. NH_3 for the series **3** and 25% aq. NH_3/EtOH (3:1) for series **2**. The DNA hairpins were subsequently purified by reversed-phase HPLC and anion-exchange HPLC. Purity and correct incorporation of the flavin and the oxetane acceptors was confirmed by analytical reversed phase HPLC and MALDI-TOF mass spectrometry (see Experimental Section).

Irradiation results and discussion: Melting-point studies of all hairpins prepared (**2a–d** and **3a–d**) confirmed that they all possess melting points well above room temperature (Figure 1). Temperature dependent CD spectra (exemplified for hairpin **3a**), as depicted in Figure 4 show that the hairpins adopt a B-like conformation which melts cooperatively upon heating.[35]

All eight hairpins were finally irradiated to induce the electron injection by the flavin. To this end they were individually dissolved in irradiation buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4) containing 9.8 mM sodium dithionite as the reducing agent. The solutions were filled into small fluorescence cuvettes stoppered with a rubber septum. Argon was bubbled through the solution in order to establish anaerobic conditions. The cuvettes were irradiated with a 1000 W xenon lamp equipped with a cooled cut-off $\lambda =$ 340 nm filter. The irradiation experiments were each performed for up to 40 min. During the irradiation time small aliquots were removed from the cuvettes and analyzed by reversed-phase HPLC. Figure 5 shows the HPLC results obtained upon irradiation of hairpin **2b** as a typical example. Clearly evident is the clean reaction of the oxetane containing hairpin to a new hairpin product. Analysis of the product confirmed that the new hairpin is a DNA strand without benzophenone cleaved off, which would be the expected product of the irradiation experiment. Control experiments established that with either no dithionite in solution or in the absence of light no oxetane cleavage is observed. These results prove that in these hairpins an electron transfer occurs upon irradiation from the reduced and deprotonated flavin to the oxetane moiety. This unit cleaves spontaneously after single-electron reduction very similar to what is proposed to happen during repair of (6–4) lesions by (6–4) photolyases and what was shown to happen in model compounds.[25,26]

In order to determine the cleavage efficiency, we integrated the peak area of the new product at each time point and plotted the relative amount of product formed against the irradiation time. Figure 6 shows the curves obtained for hairpins **2a** and **2b** as examples.



Scheme 2. Synthesis of the oxetane acceptor **3**. a) benzyl bromoacetate, K_2CO_3, DMF, RT, 0°C → RT, 20 h, 68%; b) *hν*, benzophenone, MeCN, RT, 5 h, 57%; c) H_2, Pd/C, EtOAc, RT, 3 h, 98%; d) propargylamine, HOBt, TBTU, NEt_3, DMF, RT, 86%; e) 5-iodo-3′,5′-di-*O*-TBDMS-deoxyuridine, CuI, $[PdCl_2(PPh_3)_2]$, DIEA, DMF, RT, 12 h, 66%; f) HF·pyridine, pyridine, THF, RT, 12 h, then MeOTMS; g) DMTCl, 4-DMAP, pyridine, mol sieves 4 Å, RT, 54 h, 41% over two steps; h) β-cyanoethyltetraisopropylphosphordiamidite, diisopropylammonium tetrazolate, dichloromethane, RT, 12 h, 58%.

d with four base pairs between the flavin head and the oxetane acceptor are consistently slower compared with the hairpins with only three base pairs. We also observe—in agreement with previous data—that the cleavage efficiency in all hairpins with GC base pairs between the flavin donor and the oxetane acceptor is significantly reduced.[14] These data support our[14] and Rokita's[15] previous observation that GC base pairs reduce the efficiency of the electron-transfer process. It is very interesting that we are able to confirm these observations with the hairpins **3a–d** in which the acceptor unit is placed far outside the duplex structure.

The results obtained with the oxetane acceptors **2** are not as consistent. This is not surprising if we consider that the oxetane

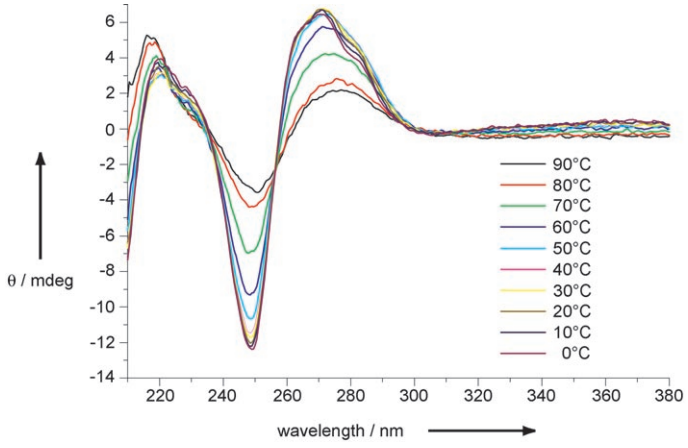


Figure 4. Temperature dependent CD spectra of the hairpin **3a**. Conditions: Hairpin concentration = 3 μM in buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4).

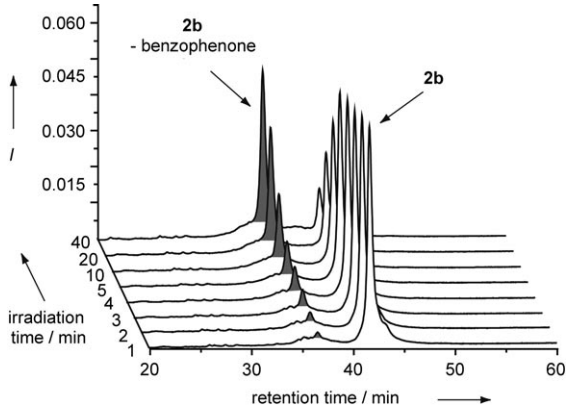


Figure 5. HPLC traces obtained during the irradiation experiment with hairpin **2b**. Assay solution: Hairpin concentration = 20 μM in buffer (150 mM NaCl, 10 mM Tris-HCl, 9.8 mM $Na_2S_2O_4$, pH 7.4). Irradiation with 1000 W xenon lamp, cut-off filter $\lambda = 340$ nm, $T = 20$°C. HPLC conditions: Nucleosil column (120 Å, 3 μm); eluting buffers (buffer A: 0.1 M $NHEt_3OAc$ in H_2O; buffer B: 0.1 M $NHEt_3OAc$ in MeCN); Gradient: 0–10% B in 10 min and then up to 45% B in 45 min at a flow of 0.5 mL min^{-1}. I = relative absorbance.

The bars in Figure 7 summarize the data on the cleavage efficiency as % reaction product formed per minute of irradiation. A first observation is that the oxetanes cleave cleanly and efficiently in all hairpins to give the expected products. The fast hairpins cleave with efficiencies of more than 10% product formation per minute.

Analysis of the hairpins with oxetane acceptor **3** shows first of all the expected small distance dependence of the electron transfer induced oxetane cleavage in accord with data from other systems.[36] The hairpins of the series **c** and close to the duplex induces a rather strong perturbation of the DNA double-helix structure. We can, however, conclude that the oxetane cleavage in these hairpins is always slower compared with series **3**. This is particularly obvious when we compare hairpins **3c** and **3d** with **2c** and **2d**. In **2d** and **3d** four strongly helix stabilizing GC base pairs separate the flavin donor and the acceptor. In these series **2c**,**d** and **3c**,**d**

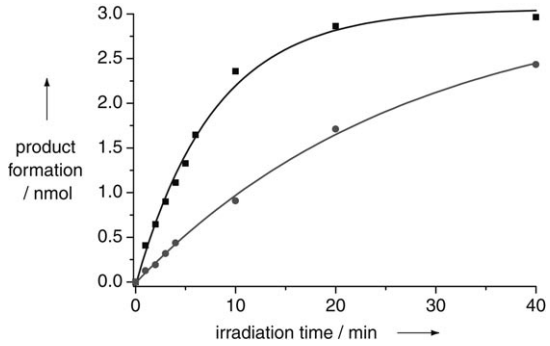


Figure 6. Time dependent formation of the product hairpins **2a** and **2b** after irradiation. Assay solution: hairpin concentration = 20 μM in buffer (150 mM NaCl, 10 mM Tris-HCl, 9.8 mM $Na_2S_2O_4$, pH 7.4). Irradiation with 1000 W xenon lamp, cut-off filter $\lambda = 340$ nm, $T = 20$ °C; ■: **2a**, ●: **2b**. Data points were fitted exponentially using the program Microcal Origin.

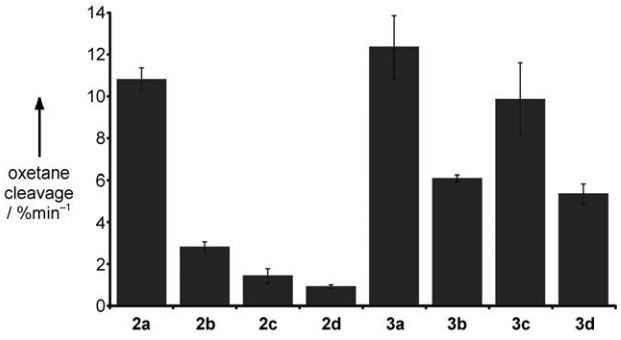


Figure 7. Bar graph representation of the irradiation data. Depiction of the efficiencies of oxetane cleavage of hairpins **2a–d** and **3a–d** in percent per minute of irradiation. Assay solution, hairpin concentration: 20 μM in buffer (150 mM NaCl, 10 mM Tris-HCl, 9.8 mM $Na_2S_2O_4$, pH 7.4). Irradiation with 1000 W xenon lamp, cut-off filter 340 nm, $T = 20$ °C. Each graph is an average of three independent measurements. The error bars were calculated from the deviation.

cleavage of the oxetane outside the helix is significantly faster. It is clear that the strong helix destruction caused by the oxetane acceptor **2** gives rise to a reduced cleavage efficiency. In contrast, placing the acceptor outside the duplex, has only a small effect.

One exception is hairpin **2a**. In this compound, cleavage of the oxetane is particularly fast. However, this hairpin features also the smallest melting temperature. We believe that the three AT base pairs separating the strongly helix disturbing oxetane **2** from the flavin cap are not able to enforce a rigid double helix structure causing, for example, slippage that brings the flavin donor closer to the oxetane acceptor.

We next analyzed if one could disrupt the electron-transfer process in series **2** and **3** with N_2O as a scavenger for solvated electrons. The hairpins **2a**,**b** and **3a**,**b**,**d** were selected for this study. All hairpins were irradiated in the presence of $Na_2S_2O_4$ once under an argon and N_2O atmosphere. In the latter experiment we bubbled N_2O through the solution for about 20 min prior to the irradiation. Analysis of the oxetane cleavage efficiencies showed (Figure 8) that the presence of N_2O has no effect! Based on this result we can fully rule out that the excess electrons move along the DNA as a solvated species. This result was confirmed in a second set of experiments in which we replaced $Na_2S_2O_4$ as the flavin reducing agent by a photoreduction process with EDTA as the photoreducing agent.[37] We had feared that the dithionite might somehow react with the N_2O. The EDTA procedure provided the reduced flavin much cleaner but required pre-irradiation. For the experiment the hairpins were dissolved in an EDTA containing buffer (150 mM NaCl, 10 mM Tris-HCl, 30 mM EDTA, pH 7.4) and irradiated for one minute with white light. After this, the flavin was fully reduced. Irradiation was then continued under the same conditions as with $Na_2S_2O_4$ and aliquots were removed for analysis. Comparing the data obtained with EDTA reduction in an argon atmosphere with those obtained in the presence of N_2O revealed, however, again no difference. The involvement of solvated electrons in the cleavage of the oxetane acceptor outside the duplex structure can be again ruled out.

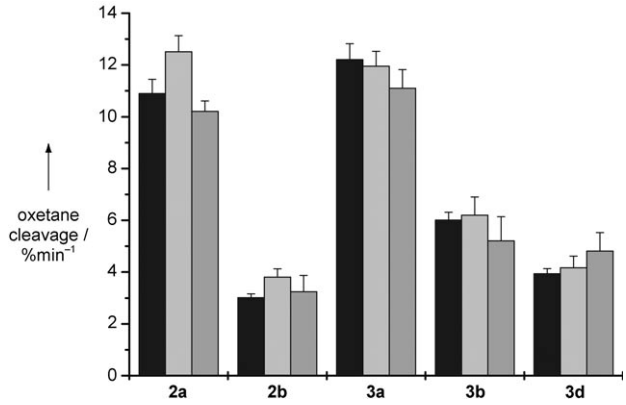


Figure 8. Bar graph representation of the irradiation data. Depiction of the efficiencies of oxetane cleavage of hairpins **2a**,**b** and **3a**,**b**,**d** in percent per minute. Assay solution, hairpin concentration: 20 μM in buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4). ■: Buffer contains 9.8 mM $Na_2S_2O_4$, Ar; ■: buffer plus 80 μL 0.2 M EDTA, 3 μL 20 mM D-(+)-mannitol, Ar; ■: buffer plus 80 μL 0.2 M EDTA, 3 μL 20 mM D-(+)-mannitol, N_2O; irradiation with 1000 W xenon lamp, cut-off filter 340 nm, $T = 20$ °C.

Conclusion

In summary we have prepared the first oligonucleotides with oxetane electron acceptors stably and side specifically incorporated. The oxetane could be cleaved in the presence of a reduced and deprotonated flavin in a light dependent process inside the DNA duplex. Similar to the repair of (6–4) lesions by the flavin containing (6–4) photolyases[38–40] the cleavage involves an excess electron transfer from the flavin to the oxetane.[25,26,28,32] The presence of a reductive cleavage mechanism is supported by the fact that the flavin was required in the reduced and deprotonated state in which it can only donate and not accept an electron. Secondly, the observed sequence and distance dependence of the cleavage

process is in accord with other systems that depend on electron donation into the duplex rather than electron abstraction and hole transfer.

Most surprising is the observation that the oxetane could be cleaved efficiently even if it was placed 10 Å away from the duplex. This fact together with the result that the cleavage in all hairpins is not diminished in the presence of N_2O, which is an efficient quencher for solvated electrons, shows that the excess electrons move through the duplex and not as a solvated electron through solvent or along the DNA backbone as previously suggested![19] If the oxetane is placed in close proximity to the center of the duplex, the DNA double helix is significantly disturbed. We observe that this helix destabilization has a larger effect on the efficiency of the reductive oxetane cleavage than placing the oxetane away from the duplex.

Experimental Section

General: Melting points are measured on a Büchi B-540 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Varian Mercury 200 (200 MHz), Bruker AMX 300 (300 MHz), Varian XL400 (400 MHz) and Bruker AMX 600 spectrometers. The chemical shifts were referenced to DMSO ($\delta = 2.50$ ppm) in [D$_6$]DMSO and $CHCl_3$ ($\delta = 7.26$ ppm) in $CDCl_3$. ^{13}C NMR spectra were recorded on Bruker AMX 300 (75 MHz), Varian XL400 (100 MHz) and Bruker AMX 600 (150 MHz) spectrometers. The chemical shifts were referenced to DMSO ($\delta = 39.43$ ppm) in [D$_6$]DMSO and $CHCl_3$ ($\delta = 77.00$ ppm) in $CDCl_3$. ^{31}P NMR spectra were recorded on Varian Mercury 200 (81 MHz). Standard pulse sequences were employed for ^{1}H 2D NOESY, ^{1}H,^{1}H and ^{1}H,^{13}C correlation studies. IR spectra were recorded with a Perkin-Elmer FT-IR spectrum 100 equipped with an ATR unit. Mass spectra and high-resolution mass spectra were measured on Finnigan MAT TSQ 700, Finnigan MAT 95, Finnigan MAT 90, Finnigan LTQ FT-ICR and Bruker Autoflex II (MALDI-TOF) mass spectrometers. Optical rotations were measured on a Perkin–Elmer 241 polarimeter.

All solvents were of the quality puriss., p.a., or purum. Purum solvents were distilled prior to use. The commercially available reagents were used as received without further purification. All reactions requiring dry conditions were carried out under nitrogen atmosphere in dried glassware.

3-Methylthymine (6):[34] Sodium hydride (1.293 g, 56.26 mmol) was added to a stirred solution of 4-thiothymine (**4**; 4.000 g, 28.14 mmol) in dry DMF (400 mL). Methyl iodide (5.26 mL, 84.40 mmol) was added dropwise and the resulting mixture was stirred at ambient temperature for 18 h. After removal of the solvent under reduced pressure, the residue was triturated with chloroform and filtered. The filtrate was concentrated under reduced pressure and the remaining solid was purified by flash chromatography (silica gel, 1% methanol/chloroform) to give the intermediate (2*S*)-methyl-3-methylthymine (**5**; 3.125 g, 65%) as a white solid. This was dissolved in 2 N HCl (200 mL) and heated to 80 °C for 8 h. The solvent was removed under reduced pressure and the residue extracted with $CHCl_3$ (300 mL). The solution was concentrated in vacuo to give **6** (2.360 g, 93%) as a white solid. All spectroscopic data were in agreement with those given in ref. [34].

Thymine oxetane 7: In a Pyrex vessel ($\lambda > 340$ nm) benzophenone (6.137 g, 33.68 mmol) was added to a solution of 3-methylthymine (**6**; 2.360 g, 16.84 mmol) in dry acetonitrile (800 mL). The resulting mixture was degassed by sparging with argon for 20 min. The solution was irradiated at 10 °C under an argon atmosphere for 2 h using a medium pressure mercury lamp (750 W). The solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel, 1% methanol/chloroform) to give **7** as a white solid (2.270 g, 44%). $R_f = 0.36$ (5% methanol/chloroform); m.p. 191 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.41$–7.23 (m, 10H), 6.35 (d, ^3J(H,H) = 4.2 Hz, 1H), 4.70 (d, ^3J(H,H) = 4.2 Hz, 1H), 2.80 (s, 3H), 1.77 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 170.3$, 152.5, 144.3, 138.5, 128.6 (2C), 128.3 (2C), 127.9 (2C), 125.8 (2C), 124.8 (2C), 90.8, 75.7, 59.7, 26.8, 23.6 ppm; IR (neat): $\tilde{\nu}$ = 3326, 2978, 1730, 1687, 1473, 1448, 1382, 1300, 1238, 1128, 1105, 978, 751, 704 cm^{-1}; HRMS (ESI$^-$): *m*/*z*: calcd for $C_{19}H_{18}N_2O_3{}^{35}Cl_1$: 357.1006; found 357.1005 $[M+Cl]^-$.

Thymidine oxetane 8: Compound **7** (500 mg, 1.55 mmol) was dissolved in dry acetonitrile (30 mL). At 0 °C sodium hydride (46 mg, 1.91 mmol) was added followed by portionwise addition of α-1′-chloro-3′,5′-di-*O*-toluoyl-ribofuranoside (756 mg, 1.94 mmol). The mixture was allowed to warm to room temperature and stirred for 4 h. After dilution with chloroform (100 mL) the mixture was washed once with sat. sodium bicarbonate and with brine, dried over sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, 7.5% ethyl acetate/toluene) to give the desired β-nucleoside **8** (290 mg, 27.7%). Additionally the α-anomer was obtained (260 mg, 24.8%). $R_f = 0.20$ (10% ethyl acetate/toluene); m.p. 169 °C; $[\alpha]_D^{26} = +52.1$ ($c = 0.026$ in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.81$–7.72 (m, 4H), 7.50–7.48 (m, 2H), 7.30–7.05 (m, 12H), 6.34 (dd, ^3J(H,H) = 5.2 Hz, 9.2 Hz, 1H), 5.30 (d, ^3J(H,H) = 6.5 Hz, 1H); 4.63 (s, 1H), 4.25 (m, 1H), 4.12 (dd, ^3J(H,H) = 3.6, ^2J(H,H) = 12.1 Hz, 1H), 3.58 (dd, ^3J(H,H) = 3.0, ^2J(H,H) = 12.1 Hz, 1H), 2.52 (s, 3H), 2.40–2.35 (m, 1H), 2.32 (s, 3H), 2.30 (s, 3H), 2.09–1.99 (m, 1H), 1.56 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 170.0$, 166.0, 165.7, 151.9, 144.3, 143.9, 142.2, 137.2, 129.6 (4C), 129.1 (4C), 128.9, 128.6 (2C), 128.0 (2C), 127.9, 126.7, 126.3, 126.2 (2C), 126.1 (2C), 103.2, 90.7, 85.2, 80.7, 76.9, 74.2, 63.5, 61.7, 35.3, 27.3, 22.0, 21.5 ppm; IR (neat): $\tilde{\nu}$ = 3438, 3064, 3035, 2990, 2924, 1722, 1686, 1611, 1448, 1410, 1373, 1271, 1201, 1179, 1101, 1019, 970, 866, 843, 752, 705 cm^{-1}; HRMS (ESI$^+$): *m*/*z*: calcd for $C_{40}H_{38}N_2O_8{}^{23}Na_1$: 674.2526; found 674.2513 $[M+Na]^+$.

Deprotected thymidine oxetane 9: Potassium carbonate (141 mg, 1.02 mmol) was added to a solution of **8** (276 mg, 0.41 mmol) in dry methanol (20 mL). The resulting solution was stirred for 3 h at room temperature. After neutralization with Amberlite IR-120 resin (2.000 g) the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica gel, 2% methanol/chloroform) to give **9** (151 mg, 84%) as a compact white foam. $R_f = 0.31$ (10% methanol/chloroform); m.p. 66 °C; $[\alpha]_D^{25} = +2.96$ ($c = 0.009$ in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.62$–7.52 (m, 4H), 7.40–7.29 (m, 6H), 7.30–7.05 (m, 12H), 6.29 (dd, ^3J(H,H) = 6.0 Hz, 8.3 Hz, 1H), 4.63 (s, 1H), 4.30 (m, 1H), 3.79 (m, 1H), 3.34 (dd, ^3J(H,H) = 3.1, ^2J(H,H) = 12.2 Hz, 1H), 3.20 (dd, ^3J(H,H) = 3.0, ^2J(H,H) = 12.2 Hz, 1H), 2.61 (s, 3H), 2.40–2.35 (m, 1H), 2.06 (ddd, ^3J(H,H) = 2.7, 6.0, ^2J(H,H) = 13.5 Hz, 1H), 1.98 (ddd, ^3J(H,H) = 6.7, 8.2, ^2J(H,H) = 13.6 Hz, 1H), 1.81 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 169.9$, 151.1, 143.2, 137.4, 128.7, 128.5, 128.3 (2C), 128.1 (2C), 126.5 (2C), 126.1 (2C), 91.3, 86.2, 85.5, 76.8, 71.4, 62.4, 62.3, 39.4, 27.1, 22.6 ppm; IR (neat): $\tilde{\nu}$ = 3022, 2927, 1718, 1672, 1463, 1448, 1417, 1374, 1293, 1202, 1149, 1095, 1052, 1020, 918, 876, 750, 706, 653, 596 cm^{-1}; HRMS (ESI$^-$): *m*/*z*: calcd for $C_{24}H_{26}N_2O_6{}^{35}Cl_1$: 473.1479; found 473.1452 $[M+Cl]^-$.

DMT-protected oxetane 10: Compound **9** (110 mg, 0.25 mmol) was coevaporated twice with dry pyridine (1 mL) and dissolved in pyridine (2 mL). After addition of molecular sieves 4 Å the solution was stirred for 4 h. 4,4′-Dimethoxytritylchloride (102 mg, 0.30 mmol) was added and the mixture was stirred for 4 h at room temperature. The solvent was removed in vacuo. The residue was purified by flash chromatography (silica gel, 1% methanol/chloroform + trace pyridine) to give compound **10** (156 mg, 84%) as a yellowish film. $R_f = 0.18$ (5% methanol/chloroform); m.p. 84 °C (decomp); ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): $\delta = 7.59$ (m, 1H), 7.41–7.32 (m, 5H), 7.24–7.10 (m, 10H), 6.94–6.92 (m, 3H), 6.73 (dd, ^3J(H,H) = 4.8 Hz, 8.8 Hz, 4H), 6.10 (t, ^3J(H,H) = 6.7 Hz, 1H), 4.36 (s, 1H), 4.01 (m, 1H), 3.77 (m, 1H), 3.71 (s, 6H), 3.10 (dd, ^3J(H,H) = 4.7, ^2J(H,H) = 9.6 Hz, 1H), 2.64 (dd, ^3J(H,H) = 1.2, ^2J(H,H) = 9.6 Hz, 1H), 2.53 (s, 3H), 2.21 (br s, 1H), 2.12–2.08 (m, 1H), 1.72–1.68 (m, 1H), 1.59 ppm (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): $\delta = 169.7$, 158.6, 151.0, 149.7, 144.4, 143.6, 138.5, 136.0, 135.6, 135.4, 130.0 (2C), 129.9 (2C), 128.1 (2C),

128.0 (2C), 127.9 (2C), 127.8 (4C), 127.6, 127.0, 125.9, 125.4, 123.8, 113.2 (4C), 91.2, 86.7, 85.5, 84.0, 73.2, 63.7, 61.4, 55.2 (2C), 38.9, 27.1, 22.7 ppm; IR (neat): $\tilde{\nu}$ = 3058, 2927, 1719, 1677, 1607, 1580, 1508, 1444, 1413, 1371, 1293, 1247, 1200, 1175, 1147, 1068, 1051, 1030, 1002, 951, 916, 876, 827, 800, 791, 746, 726, 706 cm^{-1};HRMS (ESI^+): *m/z*: calcd for $C_{45}H_{44}N_2O_6{}^{23}Na_1$: 763.2995; found 763.2983 $[M+Na]^+$.

Oxetane phosphoramidite 2: Compound **10** (130 mg, 0.17 mmol) was dissolved in degassed tetrahydrofuran (1 mL). After addition of diisopropylethylamine (130 μL, 0.74 mmol) the solution was cooled to −78 °C and β-cyanoethyltetraisopropylphosphordiamidite (77 μL, 0.29 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred for 4 h. The solvent was removed under reduced pressure. The residue was purified by flash chromatography (deactivated silica gel, 1% methanol/chloroform + trace pyridine) to give **2** (110 mg, 67%) as a yellowish film. R_f = 0.29 (2% methanol/chloroform); ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 7.54–7.53 (m, 1H), 7.43–7.20 (m, 15H), 6.99–6.92 (m, 3H), 6.81–6.77 (m, 4H), 6.10 (dd, ^3J(H,H) = 5.6 Hz, 6.4 Hz, 1H), 4.43 (s, 1H), 4.23 (m, 1H), 4.12 (m, 1H), 3.72 (s, 6H), 3.71–3.51 (m, 5H), 2.96 (dd, ^3J(H,H) = 5.0, ^2J(H,H) = 9.9 Hz, 1H), 2.69 (m, 1H), 2.62 (s, 3H), 2.58 (t, ^3J(H,H) = 6.2 Hz, 2H), 2.29 (dd, ^3J(H,H) = 5.2, ^2J(H,H) = 13.2 Hz, 1H), 1.64 (s, 3H), 1.26–0.96 ppm (m, 12H); ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 169.8, 158.6, 151.2, 144.5, 143.7, 138.7, 135.8, 135.5, 130.1 (2C), 130.0 (2C), 128.1 (2C), 128.0 (2C), 127.9 (2C), 127.8 (2C), 127.6, 127.5, 126.9, 125.9 (2C), 125.4 (2C), 117.5, 113.1 (2C), 113.0 (2C), 91.2, 86.4, 85.9, 84.6, 74.5, 74.3, 63.2, 61.0, 58.1, 55.2 (2C), 43.3, 43.2, 37.5, 29.7, 27.1, 24.6, 24.5 (2C), 22.6, 20.3 ppm; ^{31}P NMR (81 MHz, $CDCl_3$, 25 °C): δ = 150.1, 149.3 ppm; IR (neat): $\tilde{\nu}$ = 3042, 2950, 2922, 1710, 1672, 1605, 1574, 1504, 1444, 1405, 1362, 1293, 1247, 1193, 1131, 1060, 1047, 1028, 998, 947, 912, 870, 821, 794, 791, 742, 720, 702 cm^{-1};HRMS (ESI^+): *m/z*: calcd for $C_{45}H_{61}N_4O_9{}^{23}Na_1P_1$: 963.4073; found 963.4059 $[M+Na]^+$.

2-Thymine acetic acid benzyl ester (12):[33] Thymine **11** (5.000 g, 39.64 mmol) and potassium carbonate (5.480 g, 39.65 mmol) were dissolved in dry DMF (120 mL). Benzyl bromoacetate (5.48 mL, 36.00 mmol) was added dropwise and the resulting mixture was stirred for 20 h at room temperature. The suspension was filtered and the solvent was removed in vacuo. The residue was treated with water (38.5 mL) and conc. HCl (1.6 mL) and stirred for 30 min. The solid was filtered off and dried under high vacuum, then crystallized from methanol to give **12** (7.300 g, 68%) as a white powder. ^{1}H NMR (200 MHz, [D$_6$]DMSO, 25 °C): δ = 11.40 (s, 1H), 7.52 (s, 1H), 7.36 (m, 5H), 5.18 (s, 2H), 4.54 (s, 2H), 1.75 ppm (s, 3H).

Thymine acetic acid benzyl ester benzophenone oxetane (13):[33] Compound **12** (7.000 g, 25.50 mmol) was dissolved in dry acetonitrile (650 mL) under sonication and benzophenone (9.320 g, 51.90 mmol) was added. The solution was transferred to a pyrex vessel and degassed by sparging with argon for 30 min. Then the solution was irradiated under argon atmosphere for 6 h with a medium pressure Hg lamp (750 W). The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (silica gel, 25% ethyl acetate/hexanes) to give **13** (6.600 g, 57%) as a white solid. ^{1}H NMR (200 MHz, [D$_6$]DMSO, 25 °C): δ = 10.48 (s, 1H), 7.20 (m, 15H), 5.21–4.97 (m, 2H), 4.97 (s, 1H), 4.28 (d, ^3J(H,H) = 17.6 Hz, 1H), 4.08 (d, ^3J(H,H) = 17.4 Hz, 1H), 1.50 ppm (s, 3H); MS (70 eV): *m/z*: 456.2 $[M^+]$.

Thymine acetic acid benzophenone oxetane (14):[33] **13** (1.000 g, 2.20 mmol) and 10% Pd/C (100 mg, 0.09 mmol) were suspended in ethyl acetate (20 mL). The suspension was stirred under H_2 atmosphere for 3 h at atmospheric pressure. After filtration through a pad of Celite the solvent was removed under reduced pressure to give **14** (790 mg, 98%) as a white solid. ^{1}H NMR (200 MHz, [D$_6$]DMSO, 25 °C): δ = 12.95 (brs, 1H), 10.43 (s, 1H), 7.52 (s, 1H), 7.23 (m, 10H), 4.88 (s, 1H), 4.24 (d, ^3J-(H,H) = 17.6 Hz, 1H), 3.85 (d, ^3J(H,H) = 17.6 Hz, 1H), 1.57 ppm (s, 3H).

Oxetane alkyne 15: HOBt (682 mg, 5.04 mmol) and TBTU (1.610 g, 4.98 mmol) were added successively to a solution of **14** (1.500 g, 4.10 mmol) in dry DMF (30 mL) and the resulting mixture was stirred for 10 min at room temperature. Propargylamine (0.53 mL, 8.21 mmol) and triethylamine (3.4 mL, 217.75 mmol) were added and the solution was stirred for another 30 min. After dilution with ethyl acetate (150 mL) it was washed three times with brine (50 mL). The combined aqueous phases were extracted three times with ethyl acetate (50 mL). The combined organic phases were dried over magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica gel, 50% ethyl acetate/hexanes) to give **15** (1.420 g, 86%) as a white solid. R_f = 0.18 (50% ethyl acetate/hexanes); m.p. 231 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 10.48 (s, 1H), 8.55 (t, ^3J(H,H) = 5.6 Hz, 1H), 7.40–7.26 (m, 10H), 4.77 (s, 1H), 4.26 (d, ^3J(H,H) = 16.6 Hz, 1H), 3.96–3.85 (m, 2H), 3.61 (d, ^3J(H,H) = 16.6 Hz, 1H), 3.15–3.12 (m, 1H), 1.59 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.6, 167.0, 151.1, 144.3, 139.4, 128.3 (2C), 128.0 (2C), 127.5, 127.4, 125.4 (2C), 124.9 (2C), 90.6, 80.7, 76.0, 73.0, 65.2, 48.1, 27.9, 23.0 ppm; IR (neat): $\tilde{\nu}$ = 3366, 3224, 3062, 2872, 1724, 1693, 1669, 1533, 1492, 1448, 1411, 1388, 1369, 1290, 1237, 1216, 980, 785, 750, 700 cm^{-1}; MS (ESI^+): *m/z*: 426.1 $[M+Na]^+$, 404.1 $[M+H]^+$; HRMS (ESI^+): *m/z*: calcd for $C_{23}H_{21}N_3O_4{}^{23}Na_1$: 426.1430; found 426.1423 $[M+Na]^+$.

TBDMS-protected oxetane deoxyuridine conjugate 16: Compound **15** (600 mg, 1.50 mmol) and DIEA (0.72 mL, 4.14 mmol) were dissolved in dry DMF (4 mL). In a second flask cuprous iodide (57 mg, 0.30 mmol), 2′-deoxy-3′,5′-di-*O*-TBDMS-5-iodouridine (728 mg, 1.25 mmol) and [Pd-$(PPh_3)_2Cl_2$] (104 mg, 0.15 mmol) were dissolved in dry DMF (4 mL). Both solutions were degassed by several freeze/thaw cycles then combined. The resulting mixture was stirred for 12 h at room temperature. The solution was diluted with ethyl acetate (50 mL) then washed with saturated aqueous ammonium chloride (30 mL) and brine (20 mL). The combined aqueous phases were extracted once with ethyl acetate (30 mL). The combined organic phases were dried over magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica gel, 40% hexanes/ethyl acetate) to give **16** (706 mg, 66%) as a yellowish solid. R_f = 0.13 (50% ethyl acetate/hexanes); m.p. 147 °C (decomp); ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 10.00 (brs, 1H), 8.40 (brs, 1H), 7.89 (s, 1H), 7.58–7.51 (m, 1H), 7.29–7.09 (m, 10H), 6.12 (t, ^3J(H,H) = 6.6 Hz, 1H), 4.80 (s, 1H), 4.42 (d, ^3J(H,H) = 16.3 Hz, 1H), 4.29–4.25 (m, 1H), 4.08–3.92 (m, 2H), 3.86–3.82 (m, 1H), 3.79–3.74 (m, 1H), 3.66–3.61 (m, 1H), 3.37–3.31 (m, 1H), 2.22–2.17 (m, 1H), 1.92–1.85 (m, 1H), 1.61 (s, 3H), 0.81 (s, 9H), 0.77 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H), −0.04 (s, 3H), −0.04 ppm (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 169.7, 167.2, 162.8, 151.8, 149.1, 143.8, 143.0, 138.7, 128.6 (2C), 128.4 (2C), 128.1, 127.9, 126.0 (2C), 125.3 (2C), 99.0, 91.1, 89.3, 88.4, 85.9, 76.4, 74.7, 72.1, 65.9, 62.8, 48.7, 42.0, 29.9, 25.9 (3C), 25.7 (3C), 23.3, 18.4, 18.0, −4.6, −4.9, −5.5, −5.5 ppm; IR (neat): $\tilde{\nu}$ = 3207, 3065, 2952, 2929, 2856, 1682, 1532, 1463, 1448, 1279, 1253, 1205, 1104, 1029, 968, 833, 777, 750, 701 cm^{-1}; MS (FAB^+): m/z: 880.7 $[M+Na]^+$, 858.8 $[M+H]^+$, 536.5, 514.6; HRMS (ESI^+): *m/z*: calcd for $C_{44}H_{60}N_5O_9Si_2$: 858.3930; found 858.3923 $[M+H]^+$.

DMT-protected oxetane deoxyuridine conjugate 18: In a polypropylene tube **16** (234 mg, 0.27 mmol) was dissolved in EtOAc (12 mL). HF·pyridine complex (Fluka, 70% HF, 0.1 mL, 3.85 mmol) and pyridine (0.1 mL, 1.02 mmol) were added and the resulting solution was shaken for 24 h at room temperature. The resulting suspension was treated with methoxytrimethylsilane (0.6 mL, 4.35 mmol, to destroy the excess HF) and shaken for another two hours. The solid was collected by centrifugation and washed twice with cold EtOAc to give crude **17**. Crude **17** was dissolved in pyridine (8 mL) and, after molecular sieves 4 Å and a catalytic amount of 4-DMAP were added, stirred for 12 h at room temperature. 4,4′-Dimethoxytritylchloride (102 mg, 0.30 mmol) was added and the mixture was stirred for another 36 h. After removal of the solvent in vacuo the crude product was purified by flash chromatography (silica gel, 0.5% pyridine/6% methanol/dichloromethane) to give **18** (103 mg, 41%) as a colourless oil. R_f = 0.15 (silica gel, 0.5% pyridine/5% methanol/dichloromethane); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 25 °C): δ = 11.67 (s, 1H), 10.47 (s, 1H), 8.57 (brs, 1H), 7.91 (s, 1H), 7.41–7.33 (m, 8H), 7.32–7.20 (m, 11H), 6.92–6.87 (m, 4H), 6.12–6.07 (m, 1H), 5.33 (s, 1H), 4.77 (s, 1H), 4.34–4.24 (m, 2H), 4.03–3.97 (m, 1H), 3.96–3.90 (m, 2H), 3.73 (s, 6H), 3.63–3.57 (m, 1H), 3.28–3.23 (m, 1H); 3.11–3.06 (m, 1H), 2.30–2.24 (m, 1H), 2.21–2.15 (m, 1H), 1.60 ppm (s, 3H); ^{13}C NMR (150 MHz, [D$_6$]DMSO, 25 °C): δ = 169.7, 166.9, 161.4, 158.0 (2C), 151.1, 149.2, 144.7, 144.3, 143.3, 139.4, 135.5, 135.1, 129.6 (2C), 129.5 (2C), 128.3 (2C), 128.0 (2C), 127.8 (2C),

127.6, 127.5, 127.4 (2C), 126.5, 125.4 (2C), 124.9 (2C), 113.1 (4C), 98.1, 90.6, 89.0, 85.7 (2C), 85.0, 76.1, 74.3, 70.3, 65.2, 63.7, 54.9 (2C), 48.1, 40.0, 28.7, 23.0 ppm; IR (neat): $\tilde{\nu}$ = 3407, 3057, 2818, 2254, 1698, 1541, 1508, 1465, 1448, 1282, 1249, 1178, 1049, 1023, 1002, 822, 759, 702 cm^{-1}; MS (ESI^+): m/z: 954.3 $[M+Na]^+$, 303.1; HRMS (ESI^+): m/z: calcd for $C_{53}H_{49}N_5O_{11}{}^{23}Na_1$: 954.3326; found 954.3329 $[M+Na]^+$.

Oxetane building block 3: Compound **18** (103 mg, 0.11 mmol) and diisopropylammoniumtetrazolate (10 mg, 0.055 mmol) were dissolved in degassed dichloromethane (2 mL). To the solution was added β-cyanoethyltetraisopropylphosphordiamidite (44 mg, 0.15 mmol). The solution was stirred for 12 h at room temperature. The solvent was removed in vacuo. The crude product was purified by flash chromatography (deactivated silica gel, 0.5% pyridine/5% methanol/dichloromethane) to give **3** (72 mg, 58%) as a colourless oil. R_f = 0.26 (silica gel, 0.5% pyridine/5% methanol/dichloromethane); ^1H NMR (600 MHz, [D$_6$]benzene, 25 °C): δ = 10.90 (br s, 1H), 8.84–8.79 (m, 1H), 8.08 (s, 1H), 7.50–7.18 (m, 20H), 6.94–6.88 (m, 4H), 6.32–6.22 (m, 1H), 4.94 (s, 1H), 4.57–4.50 (m, 1H), 4.36–4.25 (m, 1H), 4.21–4.02 (m, 3H), 3.94–3.20 (m, 8H), 3.72 (s, 6H), 2.99–2.93 (m, 1H), 2.87–2.78 (m, 1H), 2.71–2.63 (m, 1H), 1.67 (s, 3H), 1.22–1.01 ppm (m, 12H); ^{13}C NMR (150 MHz, [D$_6$]benzene, 25 °C): δ = 170.1, 167.7, 163.3, 159.3 (2C), 152.7, 149.9, 145.1, 143.2 (2C), 140.0, 136.2, 136.0, 130.6 (2C), 129.8 (2C), 128.9 (2C), 128.7 (2C), 128.5, 128.5 (2C), 128.1, 127.9 (2C), 127.4, 126.7 (2C), 125.9 (2C), 119.9, 113.9 (4C), 99.7, 91.5, 90.0, 87.4, 86.3, 86.2, 77.1, 75.1, 73.5, 66.8, 63.6, 58.6, 55.1 (2C), 49.3, 43.5, 43.4, 40.4, 30.1, 25.8, 24.6 (2C), 24.5 (2C), 23.6 ppm; ^{31}P NMR (81 MHz, C_6D_6, 25 °C): δ = 149.6, 149.5 ppm; IR (neat): $\tilde{\nu}$ = 3198, 3062, 2980, 2837, 1693, 1609, 1510, 1464, 1448, 1281, 1249, 1207, 1179, 1079, 1030, 1003, 977, 828, 754, 703 cm^{-1}; MS (ESI^-): m/z: 1193.4 $[M+NO_3]^-$, 1166.4 $[M+Cl]^-$, 1130.5 $[M-H]^-$; HRMS (ESI^-): m/z: calcd for $C_{62}H_{65}N_7O_{12}P_1$: 1130.4434; found 1130.4505 $[M-H]^-$.

X-ray crystallographic study of compound 8: A single crystal suitable for X-ray analysis was obtained by slow crystallisation from chloroform at ambient temperature.

X-ray data: Empirical formula: $C_{40}H_{38}N_2O_8$; M = 674.72 $g\,mol^{-1}$; crystal size: (0.33 × 0.43 × 0.53) mm^3 as colourless plate; monoclinic; space group $P2_1(6)$; unit cell dimensions: a = 10.855(2), b = 14.361(3), c = 11.353(2) Å; β = 100.52(3)°; V = 1740.0(6) $Å^3$; Z = 2, ρ = 1.288 $g\,cm^{-1}$; $\mu(Mo_{K\alpha})$ = 0.090 mm^{-1}; empirical absorption correction with ψ scans yields T_{min} = 0.9154 and T_{max} = 0.9980; $F(000)$ = 712.

Data collection and analysis: NONIUS-CAD-4-Diffractometer, λ = 0.71073 Å, T = 22(2) °C, ω-2Θ scan, Θ range: 2.78–25.57°; 7030 reflections collected, 3315 independent reflections, 2752 observed reflections ($I > 2\sigma I$).

Structure solution and refinement: Solution with SHELXS-90-program system;[41] refinement of 451 variables with SHELXL-97[42] converged at $R1[F^2]$ = 0.0363 [$wR2(F^2)$ = 0.0910] for 2752 data ($I > 2\sigma I$) and $R1[F^2]$ = 0.0468 [$wR2(F^2)$ = 0.1003] for all data, largest difference peak and hole 0.177 and −0.146 e $Å^{-3}$.

CCDC-289066 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

DNA synthesis and purification: Solid-phase DNA synthesis was carried out on a Perseptive Biosystems Expedite 8900 Synthesizer using Ultramild bases and reagents (Glen Research) and following standard phosphoramidite protocols. Coupling times for building blocks **2** and **3** were increased to 10 and 15 min, respectively. Oligonucleotides comprising building block **2** were cleaved from the solid support and deprotected using conc. ammonia/ethanol 3:1 for 24 h at room temperature. For hairpins containing **3** cleavage and deprotection was carried out with conc. ammonia for 3 h at 30 °C due to the base lability of the amide moiety. Analytical and preparative HPLC was performed with a Merck LaChrom system using 3 μ or 5 μ C_{18}-reversed phase columns by Macherey-Nagel and 0.1 M $NHEt_3OAc$ in water: acetonitrile as eluent. After concentration in vacuo the strands were desalted on Waters SepPak-C_{18} cartridges and concentrated again. The final DNA concentration was estimated by UV absorption measured on a Cary 100 UV/Vis spectrometer following standard procedures. The strands were further characterized by MALDI-TOF-MS. MALDI mass spectra were recorded on a Bruker Autoflex II mass spectrometer using 3-hydroxypicolinic acid as matrix substance and measuring in negative polarity mode. Table 1 shows the calculated and experimentally determined molecular masses for the investigated oligonucleotides.

Table 1. Molecular masses of the synthesized DNA hairpins (matrix: 3-hydroxypicolinic acid, negative polarity mode).

Oligonucleotide	m/z calcd $[M-H]^-$	m/z found $[M-H]^-$
2a	5535.1	5537.4
2b	5538.1	5542.7
2c	6152.3	6155.4
2d	6156.2	6159.9
3a	5726.2	5729.8
3b	5729.2	5732.8
3c	6343.3	6348.4
3d	6347.3	6351.9

Melting point experiments: UV melting points were measured on a Cary 100 UV/Vis spectrometer using 1 mL quartz cuvettes with 1 cm path length. The samples contained 150 mM NaCl, 10 mM Tris-HCl (pH 7.4) and 3 μM of the oligonucleotide. For every strand five temperature cycles from 85 °C to 0 °C were recorded, the average melting point was calculated computationally using Microcal Origin.

CD experiments: CD spectra were recorded on a Jasco J-810 spectropolarimeter equipped with a Peltier temperature controller using 2 mL quartz cuvettes with 1 cm pathlength. The samples contained 150 mM NaCl, 10 mM Tris-HCl (pH 7.4) and 3 μM of the oligonucleotide.

Irradiation experiments: In a typical irradiation experiment a 20 μM solution of the oligonucleotide containing 150 mM NaCl and 10 mM Tris-HCl (pH 7.4, total volume 150 μL) was transferred to a 1 mL quartz fluorescence cuvette sealed with a rubber septum. The solution was degassed by sparging with argon for 20 min. For reduction of the flavin two different procedures were used. For one part of the experiments 15 μL of an alkaline solution of sodium dithionite (87 mg dithionite in 5 g water + 75 μL 5 N NaOH, pH ~8) was added. Alternatively an EDTA-mediated photoreduction was used. For this purpose 80 μL EDTA 0.2 M was added and the solution pre-irradiated under anaerobic conditions for 1 minute with white light to photoreduce the flavin (monitored by fluorescence spectroscopy). After reduction we further irradiated the samples at 20 °C with a Thermo Oriel 1000 W Xe-lamp, equipped with a cooled 340 nm cut-off filter. For solvated electron quenching experiments the solution was saturated with N_2O prior to irradiation, a slow stream of N_2O was maintained throughout the experiment and 3 μL 20 mM D-mannitol was added to capture putative OH radicals. For the analysis 10 μL aliquots were removed from the assay solution after defined time intervals, aerated for 30 min and analysed. Analysis of the data was performed by HPLC (3 μ C_{18}-reversed phase columns by Macherey-Nagel and 0.1 M $NHEt_3OAc$ in water: acetonitrile as eluent) and MALDI-TOF-MS.

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