Electron Transfer in DNA from Guanine and 8-Oxoguanine to a Radical Cation of the Carbohydrate Backbone

Eric Meggers, Adrian Dussy, Thomas Schäfer, and Bernd Giese*[a]

Abstract: Photolysis of a 4′-pivaloyl-substituted nucleotide in single- and double-stranded DNA (**1**) generated an enol ether radical cation **4** that was reduced to enol ether **17** by electron transfer from the nearest guanoside (G). Variation of the nucleotide sequence demonstrated a strong distance dependence of this electron-transfer rate with $\beta = 1.0 \pm 0.1$ Å^{-1}. When 8-oxoguanosine (G^{oxo}) was used as the electron donor, the rate of the electron transfer increased by a factor of 4 but the distance dependence of the transfer remained unchanged within experimental error. In single strands, the number of intervening A, T, and C nucleotides had a much smaller effect; the rate remained nearly constant for two, three, or four intervening nucleotides. This is explained by the flexibility of the single-stranded oligonucleotides.

Keywords: DNA oxidation · DNA radicals · electron transfer · oxoguanine · radical ions

Introduction

Studies of electron transfer (ET) through DNA over the past few years have given contradictory results and started a controversy.[1] Depending upon the experimental probe, either a minute or a substantial influence of distance on the rate of ET through DNA double strands was observed.[2, 3] Recently we suggested a hopping mechanism for long-range charge transport in DNA which could help to understand the apparent discrepancy between long-range and strongly distance-dependent charge transfer in DNA.[4] According to our hopping mechanism, charge is transported over long distances by several consecutive tunneling steps.

Here we describe an assay that enables the investigation of single ET tunneling processes. In the course of the spontaneous strand scission induced by 4′-DNA radicals **2**, a radical cation **4** is generated as a reactive intermediate.[5–7] Recently, we have shown that this radical cation can be reduced by adjacent guanosides but not by the three other natural nucleosides.[8] On the basis of the analysis of products, the rates of this charge transfer (CT) step could be determined. We now present a complete and quantitative investigation of the anaerobic C-4′ radical-induced DNA strand cleavage with a focus on the formation and reaction of the intermediate radical cation **4**.

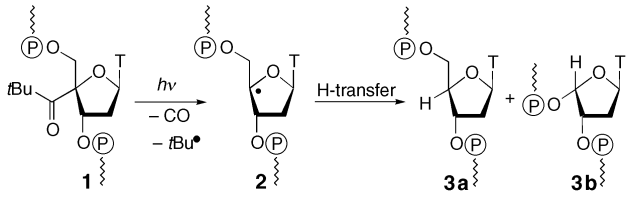
[a] Prof. Dr. B. Giese, Dr. E. Meggers, Dr. A. Dussy, Dr. T. Schäfer
Department of Chemistry, University of Basel
St. Johanns-Ring 19, CH-4056 Basel (Switzerland)
Fax: (+41) 61-267-1105
E-mail: giese@ubaclu.unibas.ch

Results and Discussion

4′-DNA radical generation: The precursor of 4′-DNA radical **2** is a DNA that contains a 4′-pivaloyl-modified strand **1** and its complementary strand.[9] The modified oligonucleotides were synthesized by solid-phase phosphoramidite chemistry. The modified DNA was photolyzed with a 500 W Hg lamp in combination with a cut-off filter (320 nm). The products were isolated by HPLC and analyzed by MALDI-ToF MS; they were quantified by dividing the peak areas by their calculated extinction coefficients and external calibration. Yields always refer to the conversion of the modified starting material **1**.

In order to determine the efficiency of 4′-DNA radical formation, we irradiated ketone **1** in the presence of an excess of glutathione diethyl ester (GSH) as H donor under anaerobic conditions.[10] At GSH concentrations of 2 mM no cleavage products were observed and the H-trapped epimers **3a** + **3b** were formed as the primary products (Scheme 1).

Products **3a** + **3b** were isolated by HPLC and identified by MALDI-ToF MS and, for one sequence, by enzymatic digestion. Digestion of **3b** showed that not only the natural nucleosides but also α-L-*threo*-thymidine were formed. Products **3a** + **3b** were formed in 87–92% yield (Table 1). Thus,



Scheme 1. Generation and trapping of 4′-DNA radical **2**.

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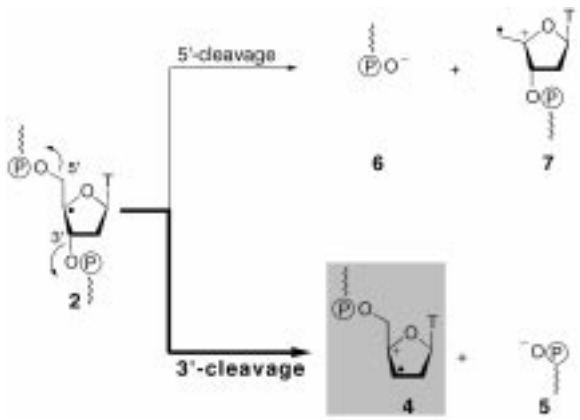
Table 1. Irradiation of 4′-pivaloyl-modified double strands with an excess of glutathione diethyl ester.[a]

Entry	Oligonucleotides[b]	**3a** + **3b** [%][c]	**3a**:**3b**
1	$C_3T_2T^*T_9$ (**1a**)	89	11.4:1
2	$TC_3TT^*T_9$ (**1b**)	87	9.7:1
3	$TG_3TT^*T_9$ (**1c**)	92	9.4:1
4	$T_4GT^*GT_8$ (**1d**)	90	5.1:1

[a] Reactions were performed in 20 mM phosphate, pH 7.0 with 100 mM NaCl at 20 °C. [b] Oligonucleotide sequences are written in the 5′ → 3′ direction; T* represents the 4′-pivaloyl-modified thymidine as in **1**. Only the modified strand of each double strand is given in the table. [c] Yields were obtained by HPLC and refer to the conversion of the modified oligonucleotide.

the Norrish I cleavage of ketone **1** is a very efficient way to generate 4′-DNA radicals **2**. The ratio **3a**/**3b** is 5:1 to 11:1 in favor of the natural epimer **3a**. This regioselectivity of the H abstraction is due to a template effect that we have described previously.[10]

Radical-induced fragmentation: In the absence of radical traps, the 4′-DNA radical **2** is expected to undergo mainly spontaneous heterolytic cleavage of the C–O bond between C-3′ and the phosphate group; this leads to radical cation **4** and 5′-phosphate **5** (Scheme 2).



Scheme 2. Competition between heterolytic cleavage of the primary and secondary C–O bonds of 4′-DNA radical **2**.

This phosphate heterolysis was originally proposed by von Sonntag and Schulte-Frohlinde[6] and could be confirmed in several model studies by our group.[7] Recently, we have determined cleavage rates of the secondary C–O bond, **2** → **4**+**5**.[10] In order to test whether the cleavage of the primary 5′-C–O bond **2** → **6**+**7** can compete to some extent with the cleavage of the secondary 3′-C–O bond, we synthesized an oligonucleotide **11** where the 4′-pivaloyl-modified thymidine is located at the 3′-end (Scheme 3). In this strand the 4′-DNA radical **12** can only undergo cleavage of the 5′-C–O bond and the cleavage rate for **12** → **6**+**13** was measured in pseudo-first-order kinetic experiments by the photolysis of a single-stranded oligonucleotide **11** in the presence of different GSH concentrations. Radical precursor **11** could be synthesized by fixing the monomer **8** on a polystyrene solid by means of **9** (**9** → **10**) and synthesizing the polymer **11** by standard phosphoramidite chemistry.[11, 12]

The plot shown in Figure 1 resulted from the photolysis of single-strand **11** in the presence of an at least tenfold excess of GSH. With a H-donor rate coefficient of $k_H = 1.0 \times 10^7\,M^{-1}s^{-1}$ for GSH,[13] a cleavage rate coefficient of $k_{cleav} = 70\,s^{-1}$ was

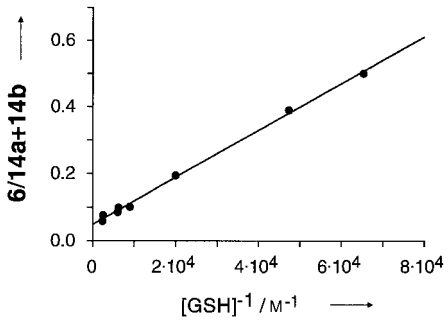
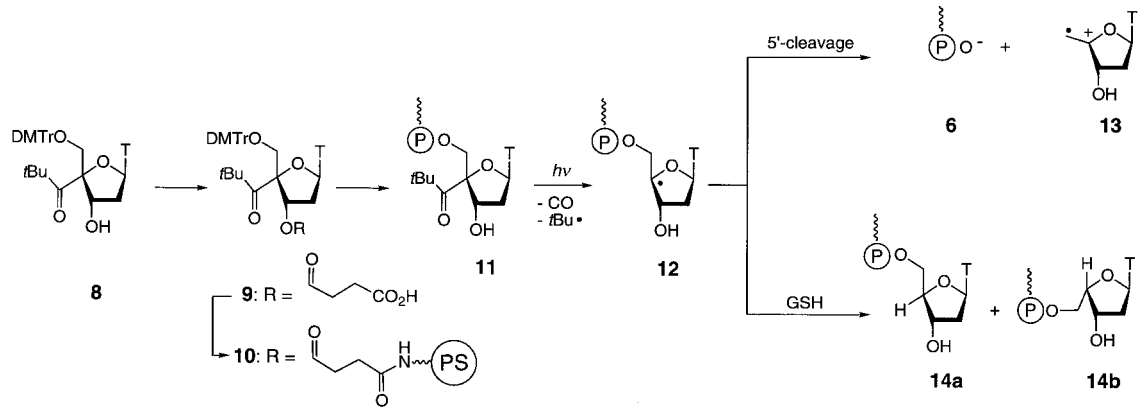


Figure 1. Ratio of the elimination product **6** to the H-abstraction products **14a** + **14b** of radical **12** as a function of the glutathione diester (GSH) concentration in buffered water solution (pH = 7.0) at 20 °C.

determined at pH 7.0. The cleavage rate coefficient of the secondary C–O bond in the reaction **2** → **4**+**5** is about $10^3\,s^{-1}$ for single strands at pH 7.0. Thus, the primary C–O bond in the reaction **2** → **6**+**7** is cleaved about fifteen times more slowly than the secondary C–O bond. This slower scission of



Scheme 3. Competition system for measuring the heterolytic cleavage of the primary C–O bond in 4′-DNA radical **12**.

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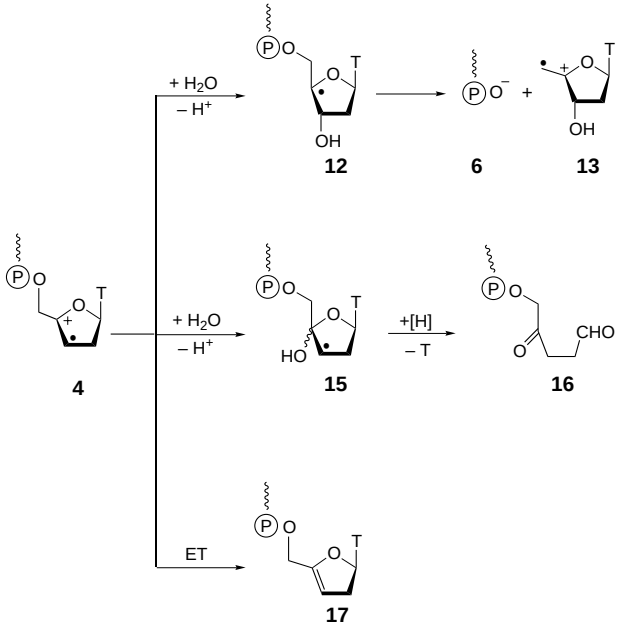
the stronger, primary C–O bond is expected and is in accordance with model studies.[7b] Thus, 4′-DNA radicals induce mainly the cleavage of the secondary C–O bond (**2** → **4**+**5**).

Water addition to the intermediate radical cation: In model studies we have shown that a radical cation of structure **4** can follow several reaction pathways (Scheme 4).[7] It can be trapped by nucleophiles like H_2O, generating new radicals **12** and **15**, or it can undergo an electron-transfer reaction that leads to enol ether **17**.



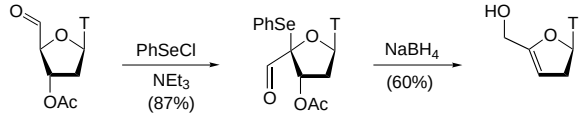
Scheme 4. Reactions of radical cation **4** with H_2O and with an electron donor.

With DNA molecules that contain only AT base pairs[8] or with **1e** where the first G is more than three base pairs away (Table 2, entry 1) no electron transfer could be observed. Irradiation of the pivaloyl-modified DNA **1e** formed the 5′-phosphate **5** in 75% yield. The 5′-phosphate **5** is the result of Norrish cleavage (**1** → **2**) and subsequent fragmentation (**2** → **4**+**5**). As demonstrated in the trapping experiments (**2** → **3a**+**3b**; see Table 1) the Norrish cleavage (**1** → **2**) occurred in different double strands (**1a–d**) in about 90% yield. Because, starting from **1e**, the overall yield of **5** is 75%, the DNA radical **2** underwent the fragmentation reaction in about 85% yield leading to **5** and the radical cation **4** in equal amounts. From the overall yield of the 5′-phosphate **5** (75%) one can conclude that radical cation **4** should have been generated also in 75% yield. This fits well with the observed 77% yield for the products **6**+**16**, which are formed by H_2O addition to radical cation **4**. The ratio **6**/**16** of 2.2 demonstrates that the regioselectivity for the H_2O addition to the oligomeric radical cation **4** is similar to that of MeOH reactions with monomeric nucleotide radical cations.[7b,f]

The identities of the 3′- and 5′-phosphorylated fragments **5** and **6** were confirmed by their MALDI-ToF mass spectra. Upon treatment with alkaline phosphatase they showed a mass shift of $m/z = 80$ in the MALDI-ToF MS, which is in agreement with the cleavage of a terminal phosphate group. In addition, for one sequence these enzymatically dephosphorylated fragments were compared with independently synthesized oligonucleotides by their HPLC retention times. Ketoaldehyde **16** was identified by MALDI-ToF MS after reduction to the diol with $NaBH_4$.

Electron transfer from guanine bases to the intermediate radical cation: DNA double strands with guanosine (G) in the vicinity of the radical cation **4** gave enol ether **17** as one of the reaction products (Scheme 4). It was identified by MALDI-ToF MS and, for one sequence, by enzymatic digestion that led to **20** as one of the monomers. Independent synthesis of nucleoside **20** was performed by phenylselenation of **18**, reduction of the aldehyde, and reductive elimination (Scheme 5).

Because enol ether **17**, the one-electron reduction product of radical cation **4**, was formed only if a guanosine residue was close to the modified thymidine in the oligonucleotide



Scheme 5. Synthesis of enol ether **20**.

Table 2. Distance dependence of product formation and the electron-transfer rates in double and single strands.[a]

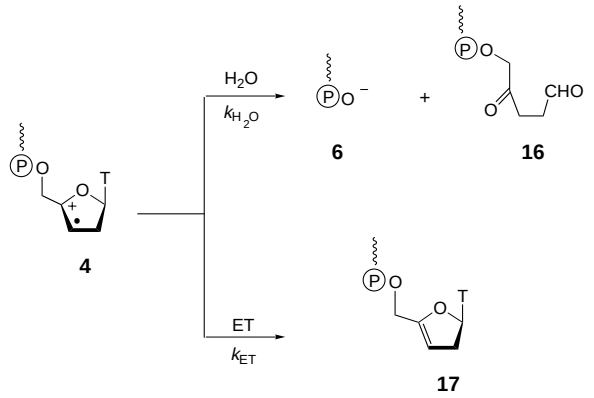
Entry	Oligonucleotide[b]	**17** [%][c,d]	**6** [%][c,d]	**16** [%][c,d]	**5** [%][c,d]	Δr [Å][e]	k_{ET} [10^6 s^{-1}][f]
1	TGCTAGCATT*TATCAGAGC (**1e**)	– (4)	53 (34)	24 (5)	75 (82)	–	– (6)
2	TGCATCTGTTT*TATCAGAGC (**1f**)	< 0.2 (6)	54 (37)	23 (3)	76 (93)	14.1	< 0.2 (7)
3	TGCTAGCATTT*TTGCAGAGC (**1g**)	0.3 (4)	52 (35)	20 (4)	71 (86)	13.2	0.3 (5)
4	TGCATCATGTT*TATCAGAGC (**1h**)	0.9 (14)	50 (30)	23 (5)	69 (82)	11.1	1.2 (20)
5	TGCTAGCACTT*TATCAGAGC (**1i**)[g]	1.6 (6)	47 (34)	22 (7)	67 (77)	10.1	2.3 (8)
6	TGCTAGCATTT*TGTCAGAGC (**1j**)	2.2 (15)	49 (30)	23 (6)	71 (85)	9.7	3.1 (21)
7	TGCTAGCTACT*TATCAGAGC (**1k**)[g]	10 (5)	48 (34)	18 (7)	74 (79)	9.5	15 (7)
8	TGCATCATTGT*TATCAGAGC (**1l**)	35 (19)	35 (27)	11 (6)	76 (75)	7.3	76 (88)
9	TGCATCTGTTT*GTACAGAGC (**1m**)	55 (47)	31 (19)	8 (2)	90 (92)	7.0	141 (104)

[a] Reactions were performed in 20 mM citrate, pH 5.0, with 100 mM NaCl at 20 °C; under these conditions the melting points (T_m) of the double strands are between 55 and 60 °C. [b] Oligonucleotide sequences are written in the 5′ →3′ direction; T* represents the 4′-pivaloyl-modified thymidine as in **1**. Only the modified strand is shown in the table. [c] Yields were obtained by HPLC and refer to the conversion of the modified oligonucleotide. [d] Results are given for double strands and single strands (in brackets). [e] Distance between radical cation **4** and the closest guanine base. [f] The electron-transfer rates were calculated from product yields as described in the text. [g] The nearest G is in the complementary strand of the double strands.

(Table 2), we concluded that G is the electron donor. Actually, guanine has the lowest oxidation potential of the four bases.[14, 15] Enol ether **17** could be detected if a G was separated from the enol ether radical cation **4** by no more than two intervening AT base pairs (Table 2, entry 3). This corresponds to a distance of 13.2 Å for the charge transfer.[16] At a distance between G and the enol ether radical cation **4** of 14.1 Å, the yield of the enol ether was so low that **17** could hardly be detected by this assay (Table 2, entry 2). The yield of **17** increased with decreasing distance between the radical cation **4** and the nearest G, and reached 35 or 55% when G was the direct neighbor of **4** (Table 2, entries 8 and 9).

The charge transfer **4** → **17** competes with the H_2O reaction that leads to 3′-phosphate **6** and ketoaldehyde **16**. The data in Table 2 demonstrate that the combined yields of the reaction products **6**+**16** + **17** of radical cation **4** were, within an error of ±2%, nearly identical to the yield of 5′-phosphate **5**. Because 5′-phosphate **5** and radical cation **4** are formed in the same reaction step, it follows that the double-stranded radical cation **4** yielded the products **6** + **16** + **17** quantitatively.

Electron-transfer rates: The competition between water addition **4** → **6**+**16** and electron transfer **4** → **17** (Scheme 6) could be used to determine relative rates for the electron transfer from guanine bases to the intermediate radical cation **4** by means of the competitive reaction kinetic procedure. This



Scheme 6. Competition between H_2O addition and electron transfer (ET) to radical cation **4**.

method can be applied if the competing reactions are first- or pseudo-first-order and the rate-determining steps are irreversible. In this case, the relative reaction rates can be deduced from the ratio of the reaction products. The intramolecular charge-transfer reaction **4** → **17** is first-order and the trapping reaction **4**+H_2O → **6**+**16** is pseudo-first-order in water as solvent. Therefore, the ratio of the products of these competing reactions is proportional to the ratio of the rate coefficient k_{ET} and the rate $r_{H_2O} = k_{H_2O}[H_2O]$. If one assumes that the rate of the trapping reaction of radical cation **4** with H_2O does not depend upon the sequence of the strands **1e–m**, then the ratio k_{CT}/r_{H_2O} is equal to the relative rate $k_{CT,rel}$ of charge transfer **4** → **17** [Eq. (1)].

$$k_{CT,rel} = k_{CT}/r_{H_2O} = \mathbf{17}/(\mathbf{6}+\mathbf{16}) \quad (1)$$

To obtain absolute rate constants for the charge transfer between radical cation **4** and guanine bases (**4** → **17**), we determined the rate of water addition to radical cation **4** (**4** → **6**+**16**) by pseudo-first-order competition kinetic experiments. The modified oligonucleotide $T_2C_3T^*T_9$ was irradiated in the presence of different concentrations of D,L-selenomethionine, D,L-methionine, and KI. These electron donors (Q) quenched radical cation **4** by ET to yield enol ether **17**. In Figure 2 the ratio (**5** – **17**)/**17** is plotted against the reciprocal concentration of electron donors.[17] For all three electron donors a

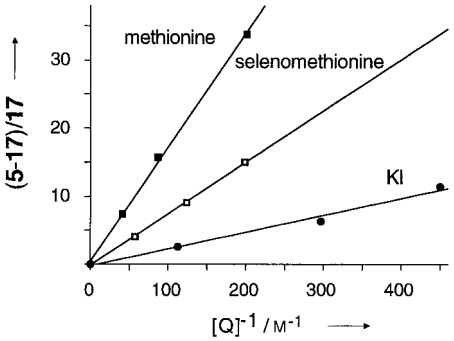


Figure 2. Influence of the concentration of different electron donors Q on the formation of products **17** and **5** from enol ether radical cation **4** in buffered (pH = 5.0) H_2O solution at 20 °C.

linear correlation was observed. The smallest slope for KI demonstrates that this is the fastest of the ET quenchers tested. If we assume that the ET between KI and radical cation **4** is nearly diffusion-controlled (ca. $5 \times 10^9\ s^{-1}$)[18] we can assign a value for the rate of water addition at radical cation **4** of $r_{H_2O} = k_{H_2O}[H_2O] = 1.1 \times 10^8\ s^{-1}$. With this value the ET rate from an adjacent guanine base to radical cation **4** can be estimated to be about $1.4 \times 10^8\ s^{-1}$ at a distance of 7 $Å^{-1}$. This rate is in accord with electron-transfer rates over 7 Å measured by Tanaka, who used a photoexcited acridinium salt as oxidant.[3e] Measurements of electron-transfer rate by fluorescence quenching often yield more than one rate for the electron transfer through DNA. This is interpreted as a result of the reactions of different conformations or, alternatively, as competing side reactions that have nothing to do with the electron transfer through DNA. In the Tanaka experiments, the fastest reaction makes up 90% of the overall process.[3e] Our study with added electron donors shows that we can exclude side reactions that do not occur from G (see Figure 2). Because our method does not tell whether the overall product formation comes from very different DNA conformations, our rate data should be considered as averaged rate data. But we believe that our assay is more uniform than most of those other systems where the electron acceptor is tethered by a linker to the DNA.

ET in double strands: The data for the ET rates in double strands **1f–m** are shown in Table 2. They demonstrate that a decrease of the distance from 13.2 to 7.0 Å increases the rate of the charge transfer by a factor of 470. A plot of these data following Equation (2)[19] gives a β value of $1.0 \pm 0.1\ Å^{-1}$ (Figure 3).

$$k_{CT,rel} \propto e^{-\beta \Delta r} \quad (2)$$

0947-6539/00/0603-0488 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 3

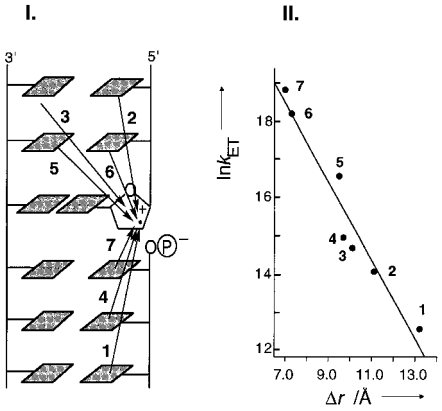


Figure 3. Distance influence of the electron-transfer rate from G to the enol ether radical cation **4**. The orientation of G with respect to the radical cation is shown in **I**. The plot for the distance dependence on the rate is given in **II**.

This β value is typical for electron tunneling by a single-step superexchange mechanism.[3, 8] The distance dependence is, for example, similar to ET reactions in proteins;[20] it can therefore be concluded that the single-step hole transfer from the radical cation of the sugar–phosphate backbone to G in DNA double strands does not benefit from base-stacking effects.

In order to address the influence of the driving force on the distance dependence of the ET step, we exchanged guanine for 8-oxoguanine (G^{oxo}) whose oxidation potential is about 0.5 eV lower than that of G.[21] Studies have demonstrated that 8-oxoguanine forms a Watson–Crick base pair with the opposite cytosine with only subtle structural perturbations in comparison with GC.[22] The data in Table 3 show that the experiments with G^{oxo}-containing oligonucleotides are less accurate than those with G-containing oligonucleotides (Table 2). The yield of 5′-phosphate **5** and the sum of the yields of the radical cation products **6** + **16** + **17** are identical only to within ±10%. This might be a result of the lability of the G^{oxo} nucleotide during photolysis and/or the workup procedure.

The yields of enol ether **17** decreased from 71% to 0.6% when the distance Δr for the ET step increased from 7.0 to 13.2 Å (Table 3, entries 1 and 8). From these yields ET rates were calculated and plotted against the distances of the ET steps according to Equation (2).[19] A linear dependence of $\ln k_{ET}$ upon the distance Δr of the ET reaction (Figure 4) with a slope of $\beta = 0.9 \pm 0.2$ Å^{-1} was observed.

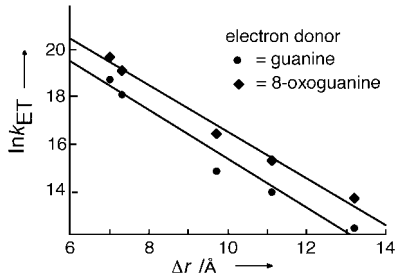


Figure 4. Distance dependence of electron-transfer rate from guanine and 8-oxoguanine to the enol ether radical cation.

In Figure 4, the corresponding data points for guanine as electron donor are shown for comparison. The line shift is the result of higher absolute ET rates (by a factor of 4 ± 1) for 8-oxoguanine as base compared to guanine. But, within experimental error, the distance dependence has not changed.

In order to learn whether the exchange of the pyrimidine base thymine for the purine base adenine as medium between the enol ether radical cation and the electron donor influences the rate, we measured the ET rates of strands **1q**, **1s**, and **1u**. Comparison of the ET rates in the double strands **1p**/**1q**, **1r**/**1s**, and **1t**/**1u** of Table 3 shows that a change from T to A has only a small influence on the ET rate.

ET in single strands: The data in brackets in Table 2 are the results for the experiments with single strands. They show that the electron-transfer rates in single strands decrease to a much smaller extent than those for double strands when the number of nucleotides between the radical cation site and the nearest G increases. Thus, on addition of one or two T nucleotides between the enol ether radical cation **4** and G, the relative charge-transfer rate decreases only slightly from 104×10^6 through 21×10^6 to 5×10^6 (Table 2, entries 9, 6, 3) or from 88×10^6 through 20×10^6 to 7×10^6 (Table 2, entries 8, 4, 2). A further increase of one or two intervening A, T, or C bases has no decreasing effect on the electron-transfer rate (Table 2, entries 1, 5, 7). Obviously, single strands are so flexible that

Table 3. Distance dependence of the product formation and the electron-transfer rates in double strands with 8-oxoguanine as electron donor.[a]

Entry	Oligonucleotide[b]	**17** [%][c]	**6** [%][c]	**16** [%][c]	**5** [%][c]	Δr [Å][d]	k_{ET} [10^6 s^{-1}][e]
1	TGCATCTGTTT*G^{oxo}TACAGAGC (**1n**)	71	16	4	70	7.0	355
2	TGCATCATTG^{oxo}T*TATCAGAGC (**1o**)	41	18	3	88	7.3	195
3	TGCTAGCATTT*TG^{oxo}TCAGAGC (**1p**)	10	41	23	60	9.7	16
4	TGCTAGCATTT*AG^{oxo}TCAGAGC (**1q**)	14	33	13	68	9.7	29
5	TGCATCATG^{oxo}TT*TATCAGAGC (**1r**)	2.3	33	17	77	11.1	4.6
6	TGCATCATG^{oxo}AT*TATCAGAGC (**1s**)	1.4	26	12	61	11.1	3.7
7	TGCTAGCATTT*TTG^{oxo}CAGAGC (**1t**)	0.6	44	19	51	13.2	0.95
8	TGCTAGCATTT*AAG^{oxo}CAGAGC (**1u**)	0.7	41	18	60	13.2	1.2
9	TGCATCTG^{oxo}TTT*TATCAGAGC (**1v**)	–	38	16	74	14.1	–

[a] Reactions were performed in 20 mM phosphate, pH 7.0 with 100 mM NaCl at 20 °C; under these conditions the melting points (T_m) are between 55 and 60 °C. [b] Oligonucleotide sequences are written in the 5′→3′ direction; T* represents the 4′-pivaloyl-modified thymidine as in **1**. Only the modified strand is shown in the table. [c] Yields were obtained by HPLC and refer to the conversion of the modified oligonucleotide. [d] Distance of the modified oligonucleotides in the 5′→3′ direction; T* represents the 4′-acylated thymine as in **1**; hybridization to the double strands was performed between radical cation **4** and the 8-oxoguanine base. [e] The electron-transfer rates were calculated from product yields as described in the text.

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conformations can be adopted in which the distance between G and the radical cation are short enough for a fast charge-transfer step, even if they are separated by several A, T, or C bases.

Conclusion

The rate of the single electron tunneling step from the nearest guanine base to the deoxyribose radical cation **4** exhibits a strong distance dependence in double strands. Although the rate depends upon the oxidation potential of the electron donor (G^{oxo} versus G), the distance dependence remains unchanged within experimental error. In single strands the number of intervening A, T, and C nucleotides has a much smaller effect on the rate of the tunneling step. Interestingly, the rate remains nearly constant if the electron donor G is separated from the enol ether radical cation by two, three, or four nucleotides.

Experimental Section

Materials: All reagents were commercially available and used without further purification. Chemicals for DNA synthesis were purchased from MWG Biotech and Glen Research. Sodium citrate (20 mM, pH 4.0, 5.0, 5.5, and 6.0) and sodium phosphate buffer solutions (20 mM, pH 7.0 and 8.5) for photolysis were obtained from Fluka (quality: for HPCE). Triethylammonium acetate (1M, TEAA) was purchased from Fluka and used for reverse-phase HPLC. Enzymes: snake venom phosphodiesterase (SVP, 2 mg mL^{-1}) was purchased from Boehringer Mannheim, calf intestinal alkaline phosphatase (AP, 1000 U mL^{-1}) from Promega and Nuclease P1 (1 mg mL^{-1}) from Pharmacia. The photolyses were performed in poly(methyl methacrylate) cuvettes from Semadeni.

Instruments: NMR spectra: Varian Gemini 300 spectrometer (^{1}H at 300 MHz, ^{13}C at 75.5 MHz). MS: VG 70-250 for FAB with 3-nitrobenzyl alcohol as matrix; Vestec Benchtop II for MALDI-ToF MS (laser wavelength 337 nm, acceleration voltage 25 kV, negative ion mode) with 2,4-dihydroxyacetophenone as matrix. UV/Vis spectrometer: Perkin Elmer Lambda II UV/Vis spectrophotometer for determining optical densities (OD) with a temperature controller for melting profiles. Extinction coefficients were calculated by the direct neighbor method.[20] HPLC: Kontron Instruments with UV detection at 260 nm and Waters Alliance with a photodiode array. HPLC columns: anion-exchange column from Macherey Nagel (ET 125/4 Nucleogen 60-7 DEAE, 125 × 4 mm), reverse-phase columns from Merck (LiChrospher 100 RP-18e, 5 μm, 250 × 4 mm) and Waters (Symmetry C18, 3.5 μm, 100 × 4.6 mm). DNA synthesizer: Expedite 8909 synthesizer from Perseptive Biosystems applying standard phosphoramidite chemistry. Photolysis: Osram 500 W mercury arc high-pressure lamp in combination with a 320 nm cut-off filter.

General procedure for the solid-phase synthesis of the 4′-pivaloyl-modified oligonucleotides: The synthesis of oligonucleotides was carried out on a DNA synthesizer in 0.2 or 1 μmol scales (500 Å controlled pore glass support). The standard method for 2-cyanoethylphosphoramidites was used, with the exception that the coupling of the modified nucleotide was extended to 15 min. With this modification there is no noticeable difference between the efficiency of coupling for this amidite and commercially available ones. Workup used standard procedures. The purity of all oligonucleotides was controlled by anion-exchange chromatography, reverse-phase chromatography, and MALDI-ToF MS.

General procedure for irradiation and subsequent HPLC analysis: Double-stranded oligonucleotides were prepared by hybridizing 1–2 nmol 4′-pivaloyl-modified strands with 1.2 equiv of the corresponding complementary strands in 100–200 μL of phosphate- or citrate-buffered solution. The samples were annealed by heating at 70 °C for 1 min and slowly cooling to room temperature. The solutions were purged with argon for 30 min and irradiated in the absence of O_2 under an argon atmosphere at 15 °C with an Osram high-pressure mercury arc lamp (500 W, 320 nm cut-off filter). Single-stranded probes were irradiated for 3–4 min, double-stranded probes for 2–3 min so that the conversion was about 60–70 %. The irradiation mixtures were analyzed without workup directly by analytical HPLC (Waters Alliance HPLC) with UV detection at 260 nm. The peak areas were divided by the calculated extinction coefficients[20] of the corresponding oligonucleotides and quantified with external calibration.

Identification of irradiation products: All fragments were isolated by HPLC and lyophilized. The masses were determined by MALDI-ToF MS and were in each case in agreement with the proposed structure. Representative for all oligonucleotide irradiations, the products **5**, **6**, and **17** from the irradiation of $T_2G_3T^*G_3T_6$ were analyzed, as was ketoaldehyde **16** from the irradiation of modified oligonucleotide **1e** as described below.

*Identification of 5′-phosphate **5** and 3′-phosphate **6***: A buffered solution (300 μL, 20 mM sodium citrate, pH 5.0, 100 mM NaCl) containing 2 nmol $T_2G_3T^*G_3T_6$ was degassed with argon for 30 min and irradiated under an argon atmosphere for 3 min with $\lambda \geq 320$ nm. The solution was incubated with calf intestinal alkaline phosphatase (1 μL of the stock solution) and incubated for 1 h at 37 °C; a 10 μL portion was desalted with filter membranes and subjected to MALDI-ToF MS. The spectra of 5′- and 3′-phosphates **5** and **6** show a mass shift compared with the MALDI-ToF MS before digestion. This shift of $\Delta = -80$ m/z is indicative of a terminal phosphate group. Additionally, in ion-exchange HPLC of the incubated solution (Macherey Nagel Nucleogen 60-7 DEAE, 125 × 4 mm, eluent A: 20 mM phosphate, pH 7.0, 20 % acetonitrile, eluent B: A + 1M KCl; gradient: 15 % to 30 % B in 25 min, then to 100 % over 25 min), two peaks shifted to lower retention times which are identical with the retention times of independently synthesized oligonucleotides T_2G_3 and G_3T_6.

*Identification of enol ether **17***: A buffered solution (300 μL, 20 mM sodium citrate, pH 5.0, 100 mM NaCl) containing 3 nmol $T_2G_3T^*G_3T_6$ was degassed with argon for 30 min and irradiated under an argon atmosphere for 3 min with $\lambda \geq 320$ nm. The solution was injected onto a reverse-phase HPLC column (Waters Symmetry C18, 3.5 μm, 100 × 4.6 mm; eluent A: 0.1M TEAA; eluent B: acetonitrile; flow rate 1.5 mL min^{-1}; room temperature) without further workup. Gradient: 6 % B for 2 min, then in 60 min to 13 % B. The enol ether **17** ($t_R = 22$ min) was isolated, lyophilized, and redissolved in phosphate buffer (400 μL, 10 mM KH_2PO_4, pH 7.0). An enzyme mixture of nuclease P1 (2 μL), snake venom phosphodiesterase (2 μL), and alkaline phosphodiesterase (2 μL) was added and the mixture was incubated overnight at 37 °C. The solution was analyzed with reverse-phase chromatography (Merck LiChrospher RP18e, 100-5, 250 × 4 mm). Eluent A: 0.1M TEAA, eluent B: acetonitrile; gradient: 3 % B for 2 min, then in 35 min to 15 % B. The chromatogram showed three peaks which were identified as thymidine (11.6 min), guanosine (10.7 min), and enol ether **20** (18.8 min) by comparison with references. Taking into account the extinction coefficients, the ratio of guanosine, thymidine, and enol ether **20** is 3:2:1, which agrees with the estimated structure of the oligonucleotide fragment **17**.

*Identification of ketoaldehyde **16***: A buffered solution (200 μL, 20 mM sodium citrate, pH 5.0, 100 mM NaCl) of 1.0 nmol 4′-pivaloyl-modified oligonucleotide **1e** and 1.2 nmol complementary strand were degassed with argon for 30 min and irradiated under an argon atmosphere with $\lambda \geq$ 320 nm for 2 min. After photolysis, $NaBH_4$ (1 mg) was added. After 2 h the mixture was quenched with KH_2PO_4 (2 mg) and injected onto a reverse-phase column (Merck LiChrospher RP18e 100-5, 250 × 4 mm; gradient: 7 % to 13 % in 30 min). Comparison of the HPLC trace before and after $NaBH_4$ treatment showed that the broad peak with t_R = ca. 14 min became narrow after reduction. This peak was isolated and the mass determined with MALDI-ToF MS. The observed mass was in agreement with that of the reduced form of ketoaldehyde **16**. Surprisingly, ketoaldehyde **16** cannot be detected by MALDI-ToF MS without preceding reduction.

Quantitative trapping of 4′-DNA radical 2 from oligonucleotides 1a–d with an excess of GSH: A buffered solution (200 μL, 20 mM phosphate, pH 7.0, 100 mM NaCl) of 2.0 nmol 4′-pivaloyl-modified oligonucleotides **1a–d** (see Table 1) and 2.4 nmol of the complementary strand (17-mer oligonucleotides with one additional T base at both ends) and 300 nmol GSH were degassed with argon for 30 min and irradiated under an argon atmosphere for 3 min with $\lambda \geq 320$ nm. The solutions were injected onto a reverse-phase column (Merck LiChrospher RP18e 100-5, 250 × 4 mm;

0947-6539/00/0603-0490 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 3

eluent A: 0.1M TEAA, eluent B: acetonitrile). Elution was carried out with a 6% to 14% linear gradient of B over 40 min with a column temperature of 50 °C. The HPLC traces show the reduction epimers **3a** + **3b** (t_R = 28–31 min), starting material **1** (t_R = 36–38 min) and the complementary strand (t_R = 22–25 min). The peak areas were divided by the calculated extinction coefficients of the corresponding oligonucleotides and quantified by means of external calibration. See Table 1 for yields.

Identification of products from trapping with glutathione diethyl ester: A buffered solution (300 μL, 20 mM citrate, pH 5.0, 100 mM NaCl) of 2.0 nmol $T_2A_3T^*T_9$ with 60 nmol GSH was degassed with argon for 30 min and irradiated under an argon atmosphere for 5 min with $\lambda \geq 320$ nm. The solution was injected onto a reverse-phase column (Merck LiChrospher RP18e 100-5, 250 × 4 mm, eluent A: 0.1M TEAA, eluent B: acetonitrile; gradient: 6% B for 2 min, then in 80 min to 15% B). Products **14a** (31.0 min), **14b** (32.8 min), **3b** (53.9 min), and **3a** (56.5 min) were isolated. The lyophilized fractions were redissolved with phosphate buffer (150 μL, 20 mM sodium phosphate, pH 7.0), treated with alkaline phosphatase (1.5 μL), snake venom phosphodiesterase (1.5 μL), nuclease P1 (1.5 μL), and $MgCl_2$ (1 μL, 1M), and incubated overnight at 37 °C. The nucleoside mixture was analyzed with reverse-phase HPLC (Merck LiChrospher). Eluent A: 0.1M TEAA, eluent B: acetonitrile; gradient: 0% B for 3 min, then in 30 min to 6% B. Products **14a** and **3a** gave only peaks resulting from thymidine (12.7 min) and deoxyadenosine (18.9 min), whereas **14b** and **3b** yielded one additional peak (10.6 min) which was identified as *lyxo*-thymidine by comparison with the retention time of an authentic sample. The ratios of the peak areas are in agreement with the proposed structure.

Distance dependence of yields of enol ether 8 from irradiation of oligonucleotides 1e–m: A buffered solution (200 μL, 20 mM sodium citrate, pH 5.0, 100 mM NaCl) of 1.0 nmol 4′-pivaloyl-modified oligonucleotide **1e–m** (Table 2) and 1.2 nmol complementary strand (24-mer oligonucleotides with 2 additional T nucleotides on both ends) was degassed with argon and irradiated under an argon atmosphere for 2 min with $\lambda \geq 320$ nm. The solutions were directly analyzed by reverse-phase HPLC (Merck LiChrospher RP18e 100-5, 250 × 4 mm; eluent A: 0.1M TEAA, eluent B: acetonitrile; flow rate 1 mL min^{-1}; column temperature 60 °C). Elution was carried out with a 7% to 13% linear gradient of B over 30 min. Under these conditions 5′-phosphate **5** (t_R = 6–8 min), 3′-phosphate **6** (t_R = 10–13 min), ketoaldehyde **16** (t_R = 12–15 min), enol ether **17** (t_R = 16–19 min), complementary strand (t_R = 22–25 min), and reactant **1** (t_R = 27–30 min) could be separated and quantified. With oligonucleotides **1i–m** small amounts of side products with high masses sometimes disturb the integration of the enol ether peak. For this reason, higher masses were separated with ion-exchange HPLC prior to quantification with reverse-phase HPLC. Therefore, the irradiated solution was injected onto a Macherey Nagel Nucleogen 60-7 DEAE 125 × 4 mm column (eluent A: 20 mM phosphate, pH 7.0, 20% acetonitrile, eluent B: A + 1M KCl; flow rate 1 mL min^{-1}; column temperature 60 °C). Gradient: 5% B for 5 min, then in 0.1 min to 50% B. The fraction between 7 and 9.5 min was isolated, the acetonitrile evaporated by lyophilization and then analyzed with reverse-phase HPLC as described above.

Irradiation in the presence of external ET donors: A buffered solution (200 μL, 20 mM citrate, pH 5.0, 100 mM NaCl) of $T_2C_3T^*T_9$ (0.3 nmol) with methionine, selenomethionine, or KI (3×10^{-3} to 3×10^{-2}M) was degassed and irradiated. The solutions were injected onto a reverse-phase column (Merck LiChrospher RP18e 100-5, 250 × 4 mm; eluent A: 0.1M TEAA, eluent B: acetonitrile). Elution was carried out with a 6% to 14% linear gradient of B over 40 min. The peak areas of 5′-phosphate **5** and enol ether **17** were weighted with their extinction coefficients and the ratio (**5** − **17**)/**17** plotted as a function of the reciprocal quencher concentration, as shown in Figure 2.

Determination of the 5′-C–O bond cleavage rate by kinetic competition experiments with glutathione diethyl ester: A buffered aqueous solution (200 μL, 20 mM phosphate, pH 7.0, 0.1M NaCl) of d(GCGAT*) (0.5 nmol) and glutathione diethyl ester (2–80 nmol) was deoxygenated by treatment with argon. The thermostated solution (20 °C) was irradiated for 5 min and analyzed directly on a reverse-phase HPLC column (flow rate 1 mL min^{-1}, 25 °C). Eluent A was 0.1M triethylammonium acetate and eluent B was acetonitrile. Elution was effected with a 4–8% B linear gradient over 15 min followed by an increase to 40% B in 10 min. The ratio **6**/(**14a** + **14b**) of cleavage to H trapping was plotted against the reciprocal H-donor concentration. Given an H-donor rate coefficient of 1.0×10^7 M^{-1} s^{-1} for glutathione diethyl ester, a cleavage rate of $k_{cleav} = 70$ s^{-1} was measured from the slope of the linear function.

Synthesis of 5′-*O*-(4,4′-dimethoxytrityl)-4′-*C*-(2,2-dimethylpropanoyl)-3′-*O*-(succinyl)thymidine (9): 4-Dimethylaminopyridine (8.3 mg, 0.067 mmol), succinic anhydride (20.3 mg, 0.203 mmol), and triethylamine (15 mL, 0.135 mmol) were added to a solution of dry 5′-*O*-(4,4′-dimethoxytrityl)-4′-*C*-(2,2-dimethylpropanoyl)thymidine (**8**, 85 mg, 0.135 mmol) in anhydrous 1,2-dichloroethane (750 mL). The reaction mixture was stirred at 50 °C for 3 h and then cooled; TLC showed the formation of a new, more polar product. The solution was further diluted with 1,2-dichloroethane (10 mL), and washed with an ice-cold aqueous solution of 10% citric acid (3 × 10 mL) and water (2 × 10 mL) in a separating funnel. The organic layer was collected and dried over anhydrous $MgSO_4$. The resulting solution was filtered, concentrated under reduced pressure, and precipitated from pentane. The solid material was filtered off and dried under vacuum at room temperature to give **9** (85 mg, 0.12 mmol) in 89% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.19 (s, 1H), 8.21 (s, 1H), 7.66 (s, 1H), 7.25 (m, 9H), 6.82 (d, J = 8.6, 4H), 6.59 (dd, J = 5.7, 9.8, 1H), 5.65 (d, J = 4.4, 1H), 3.78 (s, 6H), 3.50 (m, 2H), 2.4–2.7 (m, 6H), 1.25 (s, 3H), 1.16 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 212.81, 175.31, 170.41, 164.28, 158.89, 158.86, 150.92, 143.45, 135.79, 134.48, 130.21, 130.14, 128.27, 128.05, 127.44, 113.33, 111.81, 95.73, 88.28, 85.24, 78.07, 68.62, 55.24, 45.40, 37.88, 29.21, 28.78, 26.00, 11.23; MS (FAB): m/z = 767 [M^++K]; anal. calcd for $C_{40}H_{44}N_2O_{11}$ (728.80): C 65.91%, H 6.09%, N 3.85%; found: C 65.59, H 6.14, N 3.54.

Addition of 9 to polystyrene solid support to give 10: Compound **9** (35 mg, 0.013 mmol) was dissolved in dioxane (300 mL) containing dry pyridine (10 mL) and *p*-nitrophenol (2 mg, 0.014 mmol). Dicyclohexylcarbodiimide (7 mg, 0.034 mmol) was added and the solution stirred for 5 h. The precipitated dicyclohexylurea was removed by filtration and the supernatant liquor was added to the polystyrene solid support (containing free amino groups) suspended in dimethylformamide (1 mL). Triethylamine (100 mL) was added and the solution was stirred overnight. The solid phase was filtered off, washed with dimethylformamide, methanol, and ether, and dried under vacuum. The remaining free amino groups were acylated with acetic anhydride (400 mL) and 4-dimethylaminopyridine (10 mg) in pyridine (3 mL). This solution was stirred for 45 min. The solid phase was filtered off, washed with dimethylformamide, methanol, and ether, and dried under vacuum. The amount of bound deoxyribonucleotide on the support was determined according to the original procedure to be 15 mg g^{-1}.

1-(3-*O*-Acetyl-4-phenylseleno-2-deoxy-α-L-*threo*-pento-5-aldo-1,4-furanosyl)thymine (19): Pyridinium trifluoroacetate (160 mg) was added to aldehyde **18** (15.7 g, 47.7 mmol) and coevaporated three times with benzene (40 mL). A solution of phenylselenyl chloride (13.7 g, 71.6 mmol) in dichloromethane (50 mL) was cooled (−78 °C) and treated under argon with triethylamine (19.9 mL, 143 mmol). Aldehyde **18** in abs. dichloromethane (20 mL) was added, and after 10 min the solution was warmed to 0 °C and stirred for 3 h. Diethyl ether (100 mL) was added to the solution and the product was filtered. The precipitate was dissolved in dichloromethane (1000 mL) and washed twice with water (300 mL). The solution was dried over magnesium sulfate and the dichloromethane removed in vacuo to yield 18.2 g (87%) of **19**. ^{1}H NMR ($CDCl_3$): δ = 9.23 (s, 1H, H-5′), 8.73 (br s, 1H, H-3), 7.66 (s, 1H, H-6), 7.52–7.53 (m, 2H, SePh), 7.31–7.53 (m, 3H, SePh), 6.78 (dd, J = 6.0, 9.4 Hz, 1H, H-1′), 5.79 (dd, J = 4.7 Hz, 1H, H-3′), 2.75 (ddd, J = 4.7, 9.5, 14.5 Hz, 1H, H-2′), 2.64 (dd, J = 6.0, 14.4 Hz, 1H, H-2′), 2.08 (s, 3H, AcO), 1.97 (s, 3H, Me-5); ^{13}C NMR ($CDCl_3$): δ = 164.1 (C_q, C-4), 151.0 (C_q, C-2), 136.3 (C_t, C6), 135.9, 129.3, 128.9 (C_t of PhSe), 126.3 (C_q of PhSe), 111.9 (C_q, C-5), 87.8 (C_q, C-4′), 87.1 (C_t, C-1′), 77.8 (C_t, C-3′), 64.2 (C_s, C-5′), 39.4 (C_s, C-2′), 12.5 (C_p, Me-5); FAB-MS (NBA + KCl): m/z = 437 [M+K]$^+$, 399 [M+1]$^+$; anal. calcd for $C_{16}H_{18}N_2O_5Se$ [297.29]: C 48.37, H 4.57, N 7.05; found: C 48.15, H 4.69, N 6.90.

1-(2,3-Dihydro-5-hydroxymethylfuran-2-yl)thymine (20): Selenoaldehyde **19** (200 mg, 460 mmol) was dissolved in ethanol (20 mL) and treated with sodium borohydride (87 mg, 2.30 mmol) under argon at −78 °C. After 15 min the solution was warmed to 0 °C and after an additional 30 min, acetone (1 mL) was added dropwise. The solvent was removed in vacuo and the residue was chromatographed on silica gel with dichloromethane/ethanol 10:1 to yield 62 mg (60%) of the product **20**. ^{1}H NMR ($CDCl_3$ with CD_3OD): δ = 7.17 (d, J = 1.2 Hz, 1H, H-6), 6.70 (dd, J = 4.1, 9.7 Hz, 1H,

H-1′), 5.04 – 5.07 (m, 1H, H-3′), 4.18 – 4.21 (m, 2H, H-5′), 3.24 (ddq, $J = 9.7$, 17.3, 2.0 Hz, 1H, H-2′), 2.86 (br s, 1H, OH), 2.52 (ddq, $J = 4.1$, 17.3, 1.5 Hz, 1H, H-2′), 1.91 (d, $J = 1.2$ Hz, 3H, Me-5); ^{13}C NMR ($CDCl_3$ with CD_3OD): $\delta = 164.3$ (C_q, C-4), 156.7 (C_q, C-4′), 150.3 (C_q, C-2), 135.1 (C_t, C-6), 111.6 (C_q, C-5), 95.5 (C_t, C-3′), 84.7 (C_t, C-1′), 56.4 (C_s, C-5′), 36.1 (C_s, C-2′), 11.9 (C_p, Me-5); FAB-MS (NBA + KCl): $m/z = 263$ $[M+K]^+$, 225 $[M+1]^+$. Because of the lability of the substance a correct elemental analysis could not be achieved.

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