# A new ground state single electron donor for excess electron transfer studies in DNA†

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**A new photoinducible single electron donor has been developed, which, when linked to thymidine, is shown to be an efficient ground state reducing agent in DNA; the donor can be activated at wavelengths where standard DNA does not absorb.**

Excess electron transport (EET) studies in DNA require the incorporation of photoinducible electron donors into DNA duplexes. So far, investigations have been performed with electron donors such as flavins,[1] naphthalene diamines,[2] stilbene diethers,[3] phenothiazines,[4] pyrenes,[5] platinum(II)-[6] or iridium-complexes,[7] which inject multiple electrons into DNA due to fast photocycling of the chromophore during the experiment. In addition, most of these donors are excited state donors, which establish a strong driving force for charge recombination after electron injection. For some time, we have been interested in finding novel ground state electron donors to insert into DNA, which (1) inject only a single electron into the duplex and (2) generate no driving force for charge recombination. The idea is to inject an electron into DNA, which is then free to travel through the duplex without feeling a retractive force. A previously investigated system had the strong disadvantage that photoinduction required a rather short initiation wavelength of $\lambda < 320$ nm, so that selective triggering of the electron injection event was difficult to achieve.[8] Here we report the development of a new nucleoside **1**, which is triggered at $\lambda > 340$ nm. The compound establishes an irreversible single electron injection event and keeps the driving force for charge recombination to a minimum. Both features—the lack of a recombination driving force and the fact that only a single electron is injected—will allow excess electron transfer reactions through DNA duplexes to be studied with very high precision, and enable cascade reactions triggered by a single electron in the DNA duplex to be characterized.[8]

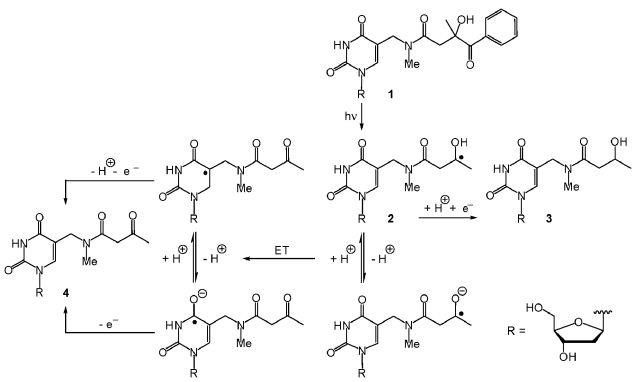
Electron transfer is established by photoinduced Norrish type I cleavage of the phenylketone **1**, which gives a highly reducing ketyl radical **2**.[8,9] This is either quenched by the surrounding medium, leading to the β-hydroxy-amide **3**, or it is oxidized by the attached pyrimidine moiety forming the β-keto-amide **4** (Scheme 1). Since the Norrish type I cleavage of the phenylketone is irreversible, electron donation occurs out of the ground state, without the possibility for charge recombination.

The synthesis of **1** was performed as depicted in Scheme 2, by coupling the TBDPS-protected thymidine methyl amine **5** with acid **6** using an HBTU/DIPEA coupling protocol, followed by deprotection of the silyl groups. Acid **6** was synthesized from the vinylbromine **8** in 4 steps (Scheme 2).

The key transformations included a palladium-*N*-heterocyclic-carbene-catalyzed carbonylative Suzuki–Miyaura coupling to give **9**[10] and a manganese-catalyzed hydro-hydroxylation[11] of the double bond in **9** to form compound **10** with the hydroxyl group inserted alpha to the carbonyl group. Phenone **10** was finally deprotected using TBAF[9] followed by a TEMPO catalyzed oxidation[8,12] of alcohol **11** to yield the final carboxylic acid **6**. For later comparison, we also prepared the expected photolysis products **3** and **4** by coupling the methylamine thymidine **5** with the corresponding carboxylic acids using an EDC/HCl/HOBt or a HBTU/DIPEA protocol.

In order to study the photochemical properties of nucleoside **1**, we dissolved the compound in $H_2O$ : MeOH (4 : 1) to a concentration of 70 μM and irradiated with a Hg(Xe) arc lamp.‡ After defined time intervals the samples were removed from the irradiated solution and analyzed by reversed phase HPLC (0–75% in 45 min, 0.1 M $NEt_3$/AcOH in $H_2O$ → $H_2O$–$CH_3CN$ 20 : 80). After irradiation for 5 min, most of ketone **1** was already cleaved (Fig. 1) showing that the Norrish type I cleavage is an extremely efficient process. After 20 min, ketone **1** could no longer be detected, indicating total conversion.

The HPLC chromatograms depicted in Fig. 1 show the detection of two new compounds absorbing at the detection wavelength of 260 nm. The compound with the shorter retention time of $t_R = 22.5$ min is clearly the major product. The




**Scheme 1** Photocleavage of single electron donor **1** to ketyl radical **2** and its conversion to the products β-keto-amide **3** and β-hydroxyl-amide **4**.

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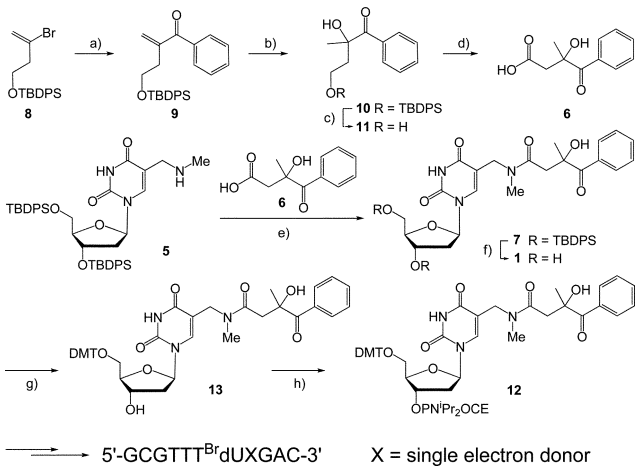
† Electronic supplementary information (ESI) available: Syntheses of compounds **1–13**, HPLC and UV chromatograms for **1–3**, details about the DNA synthesis. See DOI: 10.1039/b906180k




**Scheme 2** Synthesis of **1** and its incorporation into DNA. (a) $PhB(OH)_2$, PEPPSI-IPr, $CsCO_3$, PhCl, CO (balloon), 80 °C, 4 h, 71% (b) $Mn(dpm)_3$, $PhSiH_3$, $O_2$ (balloon), $P(OEt)_3$, $^i$PrOH, 0 °C, 2 h, 64% (c) TBAF, THF, 25 °C, 20 min, 86% (d) TEMPO, $NaClO_2$, NaOCl, buffer (pH 6.8), $CH_3CN$, 35 °C, 24 h, 88% (e) HBTU, DIPEA, DMF, 45 °C, 20 h, 81% (f) HF, pyridine, 22 °C, 18 h, 98% (g) DMT-OTf, 3 Å MS, pyridine, 22 °C, 5 h, 89% (h) $P(N^iPr_2)_2OCE$, $^iPr_2NH_2$-tetrazolate, $CH_2Cl_2$, 0 °C, 2.5 h, 75%.

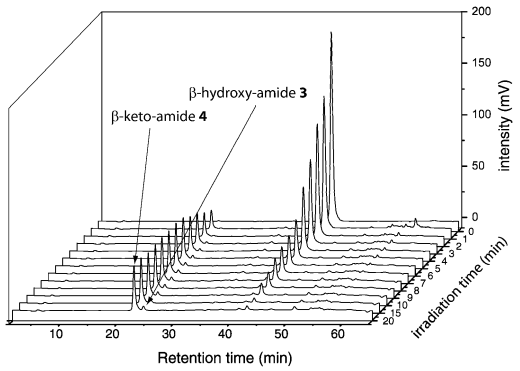



**Fig. 1** HPLC chromatograms at 260 nm showing the time dependency of the photocleavage of donor **1** (42.3 min) to β-keto-amide **4** (22.5 min) and β-hydroxy-amide **3** (24.2 min).

minor product has a retention time of $t_R$ = 24.2 min. In order to study if the major product is compound **4**, generated by quenching of the ketyl radical **2** by electron donation to the dT-base, or compound **3**, which is expected to form after $H^\bullet$ ($H^+ + e^-$) transfer from the solvent (Scheme 1), we co-injected these compounds along with the irradiation mixture into the HPLC and measured mass spectra of the HPLC peaks. The result of this study was that irradiation of compound **1** led predominately to formation of compound **4** due to electron transfer to the thymine base.

To show that the electron donor **1** can be inserted in oligonucleotides, we prepared the phosphoramidite **12** from **7** by standard procedures. Solid phase synthesis was used to generate a DNA strand 5′-GCGTTT$^{Br}$dUXGAC-3′ featuring 5-Br-dU ($^{Br}$dU) as an electron acceptor next to the donor **1** (X). Detailed analysis of the DNA strand showed that compound **1** can be safely incorporated, however, ultra-mild deprotection (0.05 M $K_2CO_3$ in MeOH) was necessary to avoid cleavage of the tertiary amide. Next we irradiated the DNA single strand to see if the injected electron is able to hop to the nearby $^{Br}$dU to cause debromination. This is indeed the case. MALDI-TOF of the irradiated single strand clearly shows the debrominated strand with a mass of 3436 Da (see ESI†).

In summary, we report the synthesis of a new nucleoside **1** and its incorporation into DNA. Upon irradiation at $\lambda > 340$ nm, **1** undergoes an efficient Norrish type I cleavage to a ketyl radical that possesses a sufficient redox potential to reduce the attached thymine, forming a thymine radical anion. Within DNA, the radical is able to reduce a nearby 5-Br-dU base and cause debromination. The advantage of the new system is that the Norrish type I cleavage occurs at long wavelengths ($\lambda > 340$ nm), so that acceptor-modified DNA strands containing nucleoside **1** can be irradiated without fear of additional excitation of either the nucleobases or any incorporated electron acceptors such as 5-Br-dU. For us, the high efficiency of the Norrish type I cleavage is surprising given the rather weak absorption of nucleoside **1** at these wavelengths ($\varepsilon_{340\ nm}$ = 37 $M^{-1}$ $cm^{-1}$). These studies show that **1** can be incorporated into DNA and the electron injection into DNA occurs efficiently.

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## Notes and references

‡ Photolysis was carried out with an ORIEL 6295H mercury(xenon) arc lamp; 1000 W, 340 nm long-pass filter (Russian C3C17). The nucleosides were dissolved in $H_2O$ : MeOH (4 : 1) to 70 μM, and prior to the irradiations the solutions were degassed by bubbling a steady stream of argon through for 40 min.

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