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# Light-activated deoxyguanosine: photochemical regulation of peroxidase activity[†,‡]

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## Abstract

Photochemical activation of a deoxyribozyme with peroxidase activity was achieved by the synthesis and incorporation of a caged deoxyguanosine.

The oxidation of organic and inorganic substrates by peroxides in a cellular context is catalyzed by peroxidases.[1] Well-studied members of this class of proteins are horseradish peroxidase, cytochrome *c* peroxidase, and glutathione peroxidase.[1] Moreover, antibodies with peroxidase activity have been developed.[2] All these catalysts are composed of a polypeptide and a metalloporphyrin cofactor, hemin and derivatives of hemin. The Sen group evolved a DNA aptamer which binds to hemin with a high affinity (0.9 μM binding constant) and, most importantly, exhibits a significant peroxidase activity.[3-5] We and others have previously reported the light triggering of a variety of DNA functions through the installation of light-removable caging groups, including RNA cleavage, DNA transcription, amplification and polymerization, and DNA binding.[6,7] Thus we became interested in the photochemical regulation of peroxidase activity through the installation of light-removable protecting groups (caging groups) on the deoxyoligonucleotide.

The peroxidase deoxyribozyme PS2.M evolved by Sen *et al*. is an 18-mer oligonucleotide of the sequence 5′-GTGGGTAGGGCGGGTTGG-3′ which is assumed to fold into the G-quadruplex structure **D1** (Fig. 1) (a complementary structure with flipped strands in the front has been postulated by Sen *et al*. as well; not shown).[4] Due to the prevalence of deoxyguanosines in **D1**, we investigated the ability to apply our NPOM caging strategy[6,8,9] to the purine base of dG thus disrupting an essential hydrogen bond. The synthesis of the caged deoxyguanosine phosphoramidite **6** (Scheme 1) commences with the synthesis of the acetyl and dimethylformamidine protected deoxyguanosine **2** from deoxyguanosine (**1**) in 61% yield. Caging of **2** with 6-nitropiperonyloxymethylene chloride (NPOM-Cl, synthesized in 4 steps from commercially available material[9]) was achieved in 79% (DBU, DMF, rt) furnishing **3**. The diol **4** was obtained in 92% yield through removal of the acetate groups with $K_2CO_3$ in MeOH. Selective tritylation of the primary hydroxy group with DMT-Cl in pyridine delivered **5** in 77% yield. The synthesis of **6** was completed through

‡Electronic supplementary information (ESI) available: Experimental procedures and analytical data for the synthesis of the caged deoxyguanosine phosphoramidite; protocols for the measurement of peroxidase activity, CD spectra, UV spectra, and DLS; DNA decaging reactions; copies of $^1$H NMR spectra for all new compounds.

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activation as the phosphoramidite under classical conditions (2-cyanoethyl-diisopropyl-chloro phosphoramidite, DCM, DIPEA, 82% yield).

The stability of **6** to DNA synthesis conditions and its rapid decaging through irradiation with UV light of 365 nm ($\varepsilon_{365}$ = 4102 $cm^{-1}$ $M^{-1}$) was first verified (data not shown). Using standard DNA synthesis conditions[6] **6** has been incorporated at several guanosine positions of the peroxidase deoxyribozyme PS2.M (**D1**) providing the caged mutants **D2–D7** (Table 1).

In order to conduct the peroxidase-catalyzed reactions, the oligonucleotide was briefly heated to 90 °C followed by a slow cooling period to induce proper folding.[3] Complexation of the hemin cofactor (1 equiv.) was achieved through incubation for 40 min at room temperature. The oxidation reactions were then initiated through the addition of an excess of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) followed by $H_2O_2$. The reaction progress was monitored by measuring the absorbance of the ABTS radical cation at 414 nm (Fig. 2).[3]

As expected, the wild type DNAzyme PS2.M (**D1**) displayed prominent peroxidase activity regardless of UV irradiation. None of the caged DNAzymes **D2–D7** exhibited catalytic activity prior to light irradiation, regardless of the position of the caged nucleotide within the DNAzyme sequence demonstrating the ability to stringently regulate DNA function through the installation of a single NPOM caging group. The decaging was achieved by irradiating the DNAzyme solutions for 5 min (365 nm, handheld UV lamp, 23 W) after the heating–cooling period, but prior to the addition of 20 KH buffer.§ After light activation, the catalytic activity of decaged DNAzymes was almost quantitatively restored as determined by calculating reaction velocities from the initial, linear slope (Table 1).

In order to further elucidate why the caging of any of the six dG positions investigated in peroxidase PS2.M inhibited its catalytic activity, we measured the structural organisation of the non-caged oligonucleotide **D1** and the caged oligonucleotides **D2–D7** by circular dichroism spectroscopy (CD).[10,11] Initial CD measurements were taken in the reaction buffer at room temperature in the absence of hemin and showed that all caged DNAzymes had substantially different secondary structures than the non-caged **D1** (Fig. 3).

We were surprised to find that **D1** did not exhibit a spectrum consistent with the anti-parallel G-quadruplex structure proposed by Sen *et al.*[4] Based on the CD spectrum shown in Fig. 3, the deoxyoligonucleotide **D1** appeared to form a mixture of various secondary structures in solution.[10] Thus, we concluded that hemin might play an important role in the formation of a stable quadruplex structure of **D1**. When CD measurements were taken after DNA incubation with hemin (Fig. 4), the spectrum of **D1** showed a significant change, consistent with a parallel rather than an anti-parallel secondary structure, having a large positive peak at 260 nm and a small negative peak at 240 nm.[12] On the other hand the caged DNAzymes **D2–D7** did not show pronounced changes in their spectra after incubation with hemin, leading to the conclusion that one caging group is sufficient to inhibit G-quadruplex formation and complexation of hemin. However, the CD spectra of caged **D4–D6** in the absence (Fig. 3) or presence (Fig. 4) of hemin showed a tendency of the oligonucleotides to form anti-parallel secondary structures, displaying a negative peak at 260 nm and a positive

§Irradiation of the DNAzymes (caged or non-caged) in 20 KH (HEPES) buffer led to a significant decrease in catalytic activity. This is probably due to the light sensitivity of HEPES leading to $H_2O_2$ formation. The increasead $H_2O_2$ concentration thus induces oxidative damage in the DNA and/or oxidation and deactivation of hemin. The same effect was observed when the DNAzymes were diluted in 20 KH buffer that was previously exposed to 365 nm UV light. Consequently, all decagings were conducted in TE buffer.[14]

peak at 290 nm. The deoxyoligonucleotide **D7** exhibited a (parallel) structure similar to **D1**, albeit not as pronounced.

At first, these observations were in contrast to findings by Heckel *et al.*,[13] who reported that the formation of a three-layer anti-parallel G-quadruplex can only be efficiently inhibited through caging of a deoxyguanosine residue at very select positions which are both located at the core of the sequence and are involved in the formation of the middle layer. In their case, DNA strands caged at the GGG triad closest to the 5′ end, or at an outer layer of the antiparallel G-quadruplex, formed a secondary structure similar to that of the non-caged DNA. However, the findings by Heckel *et al.* are based on a slightly different oligonucleotide sequence and a different caging strategy, the installation of an *ortho*-nitrophenylpropyl group on the O-6 position of deoxyguanosine.[13]

CD spectra of the oligonucleotides **D2–D7** after decaging through UV irradiation (5 min, 365 nm, 23 W) followed by incubation with hemin showed that the DNAzymes reform the same secondary structure as observed for **D1** (Fig. 5). This is in agreement with the previously observed complete restoration of catalytic activity through irradiation.

The results of the CD measurements led to the conclusion that the initially proposed G-quadruplex structure[4,5] was not the predominant **D1** secondary structure found under our experimental conditions. Instead of a triple-layer antiparallel G-quadruplex structure, the obtained CD spectra rather are consistent with a parallel G-wire structure proposed by Ohmichi *et al.* based on CD measurements of a very similar deoxyoligonucleotide sequence (Fig. 6).[12] A parallel G-wire structure is also in agreement with our finding that the caged oligonucleotide **D2** does not adopt the same secondary structure as **D1** even though the caging group is installed on a deoxyguanosine which is not involved in G-quadruplex formation (Fig. 1). Moreover, we conducted dynamic light scattering (DLS) experiments with **D1** and **D5**. These measurements clearly indicate a substantial particle size difference between the two DNA oligomers thus suggesting a highly aggregated species for **D1** and a monomeric species for **D5** (see Supplementary Information‡). Thus, it can be hypothesized that the active structure of PS2.M (**D1**) at CD and DLS measurement concentrations (1.0 μM and 5.0 μM, respectively) is a parallel G-wire structure stabilized by hemin intercallation (Fig. 6).

Further validation that the caged deoxyribozymes **D2–D7** did not bind to hemin came from UV–vis measurements (Fig. 7). Solutions of caged DNAzymes **D2, D4** and **D7**, incubated with hemin, showed no change in the characteristic Soret band (398 nm) as compared to free hemin, while **D1** complexed with hemin exhibited a red shift (404 nm) in addition to an increased absorbance.[3] Gratifyingly, decaging (5 min, 365 nm, 23 W) enabled hemin intercallation and restoration of the wild-type structure, since the UV–vis spectra displayed the same shift in the Soret band as **D1**.

In summary, the synthesis of a photocaged deoxyguanosine phosphoramidite using our NPOM caging strategy, and its incorporation into DNA *via* standard DNA synthesis conditions was accomplished. The developed approach was then applied to the light-triggered activation of a deoxyribozyme with peroxidase activity, and excellent ‘off’ (before irradiation) and ‘on’ (after irradiation) rates were observed. The obtained results demonstrate that in order to disturb the formation of a parallel G-quadruplex (G-wire), the introduction of a single NPOM-caged dG at an arbitrary position within the DNA sequence

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is sufficient. We believe that the caged guanosine phosphoramidite **6** will find broad application in the photo-chemical regulation of DNA function.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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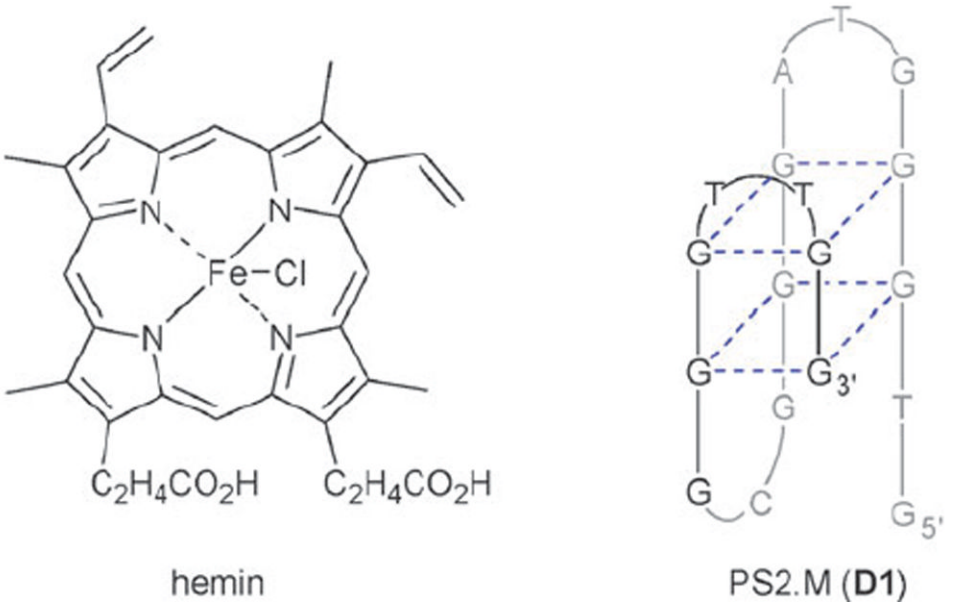



**Fig. 1.**
Structure of hemin and schematic of one of the G-quadruplex structures proposed for PS2.M (**D1**).

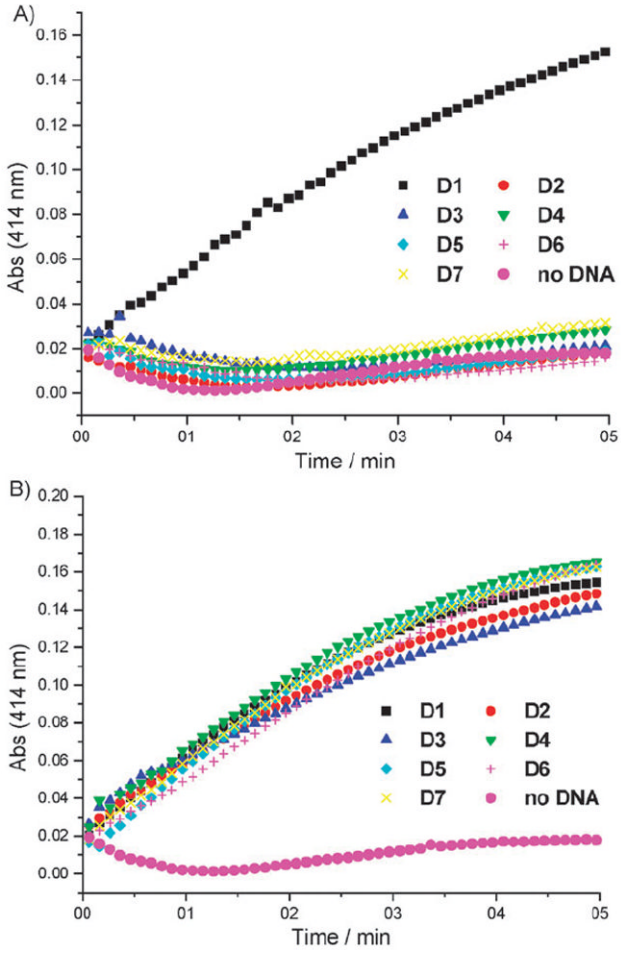



**Fig. 2.**

Oxidation of ABTS to the corresponding radical cation by the wild type DNAzyme **D1**, the caged DNAzymes **D2–D7**, and just hemin in the absence of any DNA (A) before and (B) after UV irradiation. Average of three experiments.

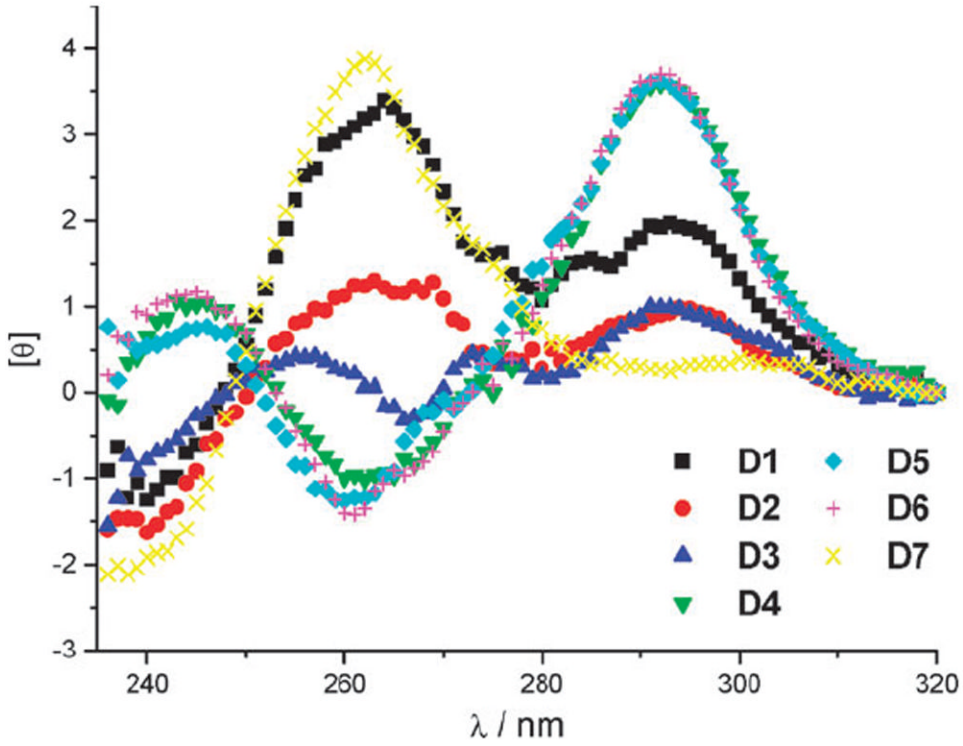



**Fig. 3.**
CD spectra of **D1** and caged DNAzymes **D2–D7** in the absence of hemin.

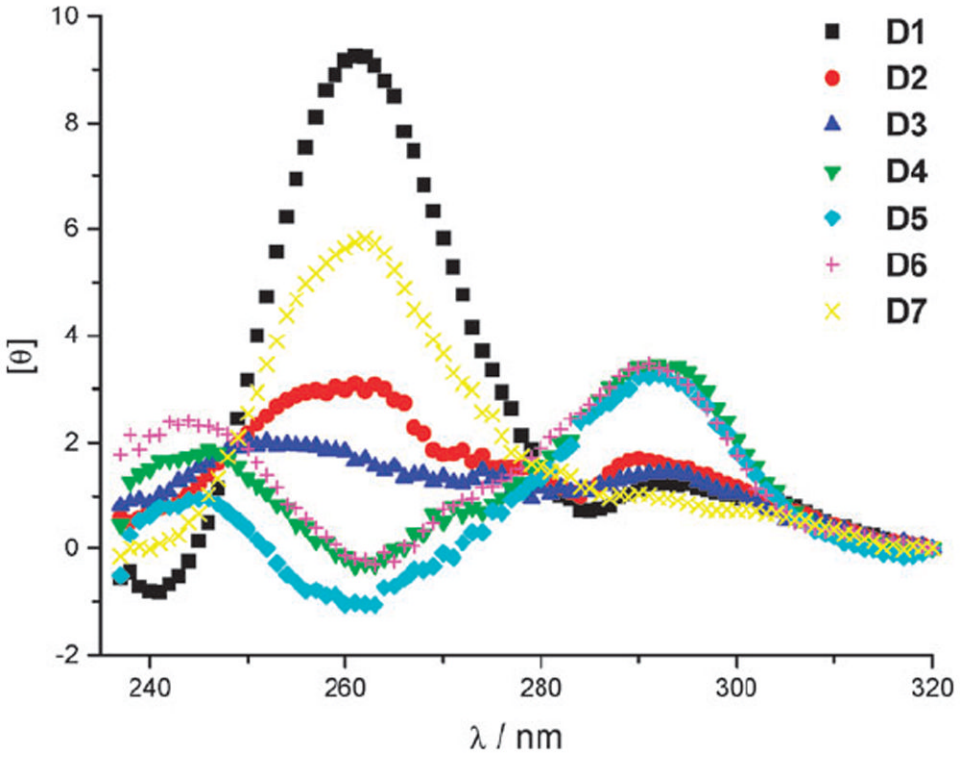



**Fig. 4.**
CD spectra of **D1** and caged DNAzymes **D2–D7** after incubation with hemin.

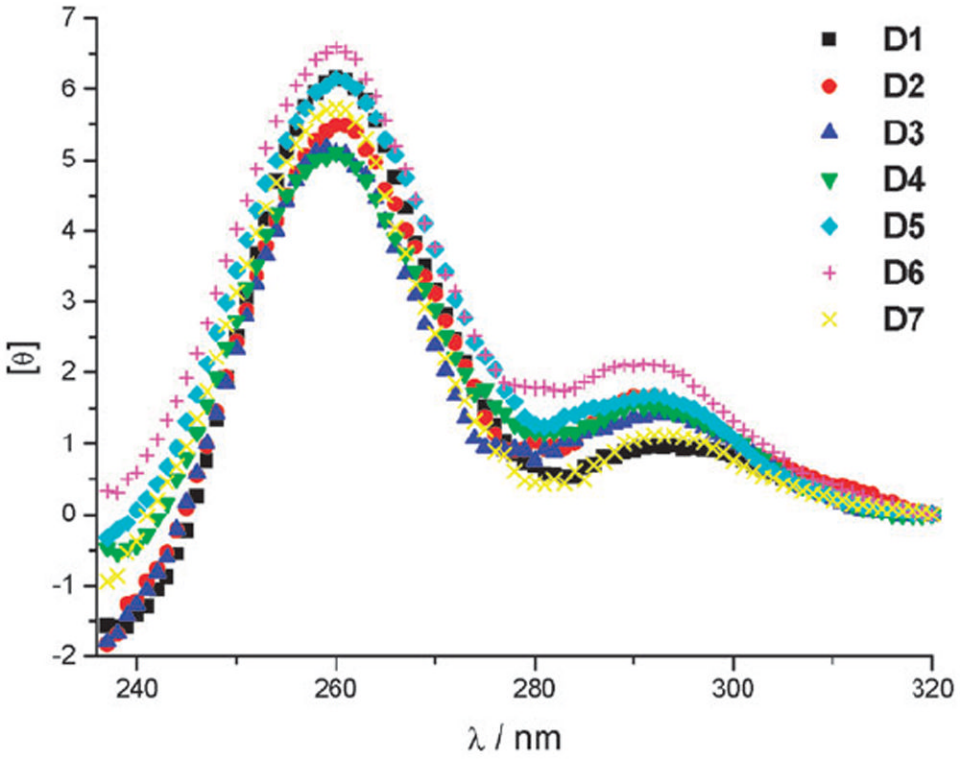



**Fig. 5.**
CD spectra of **D1** and irradiated (decaged) DNAzymes **D2–D7** after incubation with hemin.

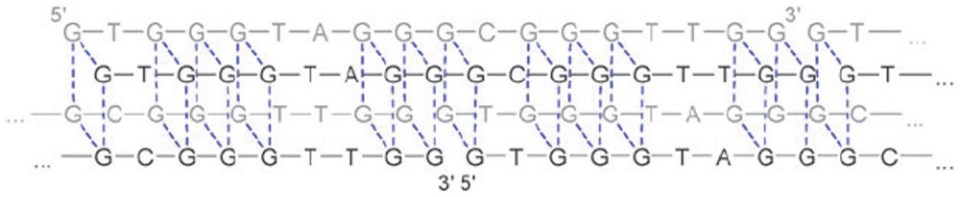



**Fig. 6.**
Proposed G-wire structure of the oligonucleotide **D1** based on CD and DLS measurements.

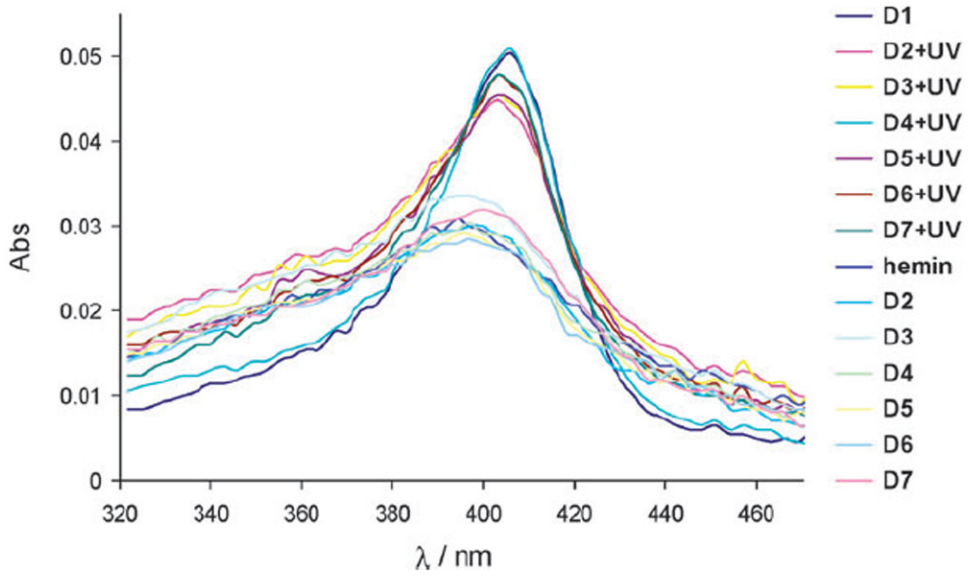
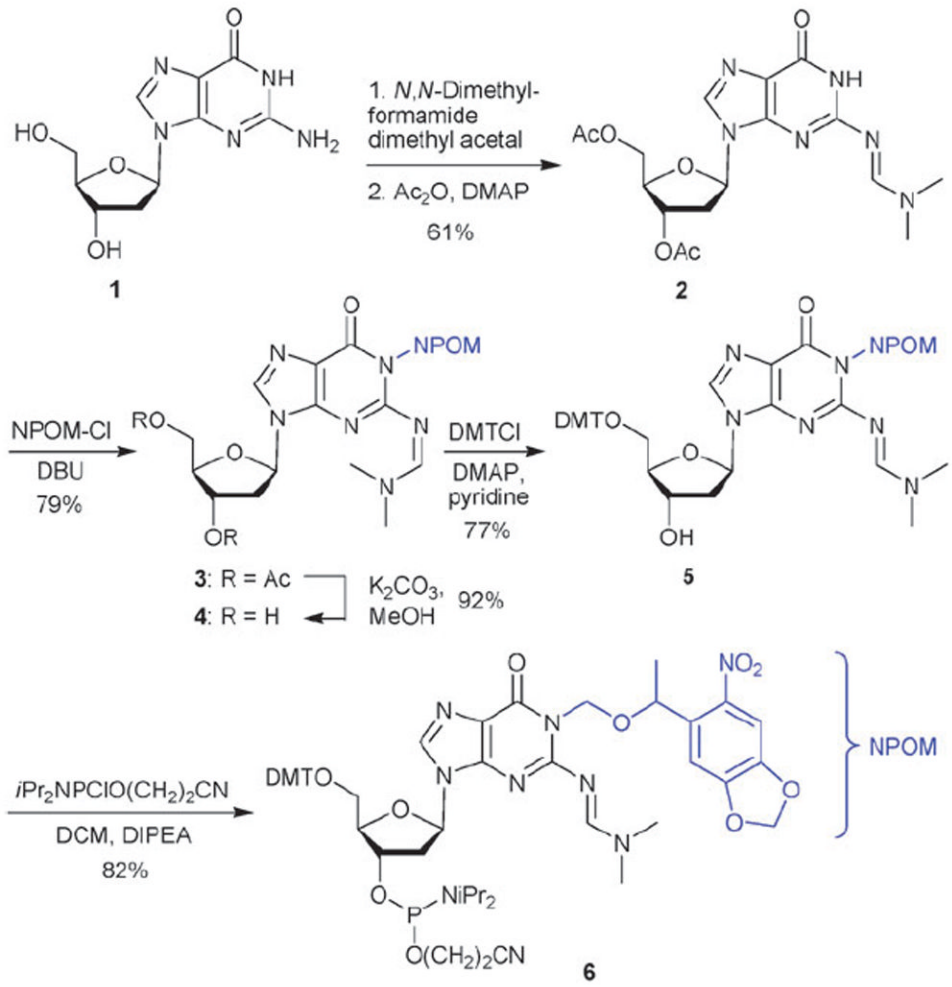



**Fig. 7.**

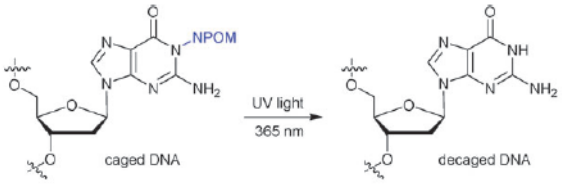
UV–vis spectra of free hemin and hemin incubated with the DNAzymes **D1–D7** with and without UV irradiation (5 μM in 20 KH buffer).




**Scheme 1.**
Synthesis of the NPOM-caged deoxyguanosine phosphoramidite **6**.

## Table 1

Synthesized peroxidase deoxyribozymes **D1–D7** and their catalytic activity before and after UV irradiation.




| | Sequence | ν(−UV) | ν(+UV) |
|---|---|---|---|
| **D1** | 5’-GTGGGTAGGGCGGGTTGG-3’ | 1.04 ± 0.02 | 1.16 ± 0.06 |
| **D2** | 5’-GTGGG*TAGGGCGGGTTGG-3’ | ND | 1.00 ± 0.04 |
| **D3** | 5’-GTGGGTAGG*GCGGGTTGG-3’ | ND | 1.18 ± 0.13 |
| **D4** | 5’-GTGGGTAGGG*CGGGTTGG-3’ | ND | 0.98 ± 0.12 |
| **D5** | 5’-GTGGGTAGGGCG*GGTTGG-3’ | ND | 1.15 ± 0.04 |
| **D6** | 5’-GTGGGTAGGG*CG*GGTTGG-3’ | ND | 0.84 ± 0.05 |
| **D7** | 5’-GTGGGTAGGGCGGGTTG*G-3’ | ND | 1.11 ± 0.09 |

G* indicates a caged deoxyguanosine.

$\nu$ = initial velocity in Abs $min^{-1}$.
ND = not detectable.