Cleavable Substrate Containing Molecular Beacons for the Quantification of DNA-Photolyase Activity

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In order to gain deeper insight into the function and interplay of proteins in cells it is essential to develop methods that allow the profiling of protein function in real time, in solution, in cells, and in cell organelles. Here we report the development of a U-type oligonucleotide (molecular beacon) that contains a fluorophore and a quencher at the tips, and in addition a substrate analogue in the loop structure. This substrate analogue induces a hairpin cleavage in response to enzyme action, which is translated into a fluorescence signal. The molecular beacon developed here was used to characterize DNA-photolyase activity. These enzymes represent a challenge for analytical methods because of their low abundance in cells. The molecular beacon made it possible to measure the activity of purified class I and class II photolyases. Photolyase activity was even detectable in crude cell extracts.

KEYWORDS:

DNA damage · enzymes · FRET · molecular beacons · proteomics

1. Introduction

Detailed analysis of the DNA, RNA, and protein content (genome, transcriptome, and proteome) of cells is of paramount importance for our understanding of the complex functional networks that control life. Knowledge gained in the areas of genomics[1, 2] and proteomics[3, 4] may lead to the discovery of new pharmaceutical targets and the development of novel therapeutic strategies.

Currently, two-dimensional gel electrophoresis or other powerful separation techniques in combination with mass spectrometry[5] are the most frequently employed methods for direct investigation of the proteomes of cells. Most recently, novel small-molecule-based techniques have been described. In this context, B. F. Cravatt coupled protease suicide inhibitors to the molecule biotin, thereby creating compounds that enabled selective extraction and quantification of proteases or other proteins with an accessible nucleophile from the complete proteome of cells (activity-based protein profiling).[6, 7] Along similar lines, P. G. Schultz reported the synthesis of constructs made up of small-molecule inhibitors linked to a peptide nucleic acid strand and a fluorophore. Addition of these compounds to a protein mixture specifically allowed "fishing" for certain proteins, which were subsequently quantified on a DNA array.[8]

In connection with our current efforts to investigate DNA repair processes, we are interested in developing methods that should in future allow quantification of DNA repair activities in the proteome of cells.

Molecular beacons are U-shaped oligonucleotides that contain a fluorophore and a quencher at their two ends (Figure 1).[9] In the closed hairpin conformation, the fluorophore and the quencher are in close proximity, which switches off the fluorescence signal by fluorescence resonance energy transfer (FRET). Opening of the hairpin separates the two chromophores, which turns the fluorescence signal on as a result of the R^{-6} dependence of the FRET on the distance R between the donor and the acceptor.[10] Since the introduction of the molecular beacon concept by F. R. Kramer and S. Tyagi in 1996,[11] such U-type oligonucleotides have been constructed to open up upon binding to, for example, fully complementary DNA[12, 13] or DNA-binding proteins.[14–16] Molecular beacons have also been used to monitor the activities of DNA-cleaving small molecules, DNA-cleaving proteins,[17–19] and self-replicating systems.[20] Today, molecular beacons have established themselves as extremely sensitive analytes with applications such as 1) quantification of PCR amplicons,[21, 22] 2) detection of genetic mutations,[23, 24] 3) clinical diagnosis of pathogenic viruses,[25] and 4) detection of mRNA[26, 27] or proteolytic enzymes[28] within living cells.

The molecular beacon strategy that we employed to quantify repair activity is schematically depicted in Figure 1. Besides the fluorophore (6-FAM; excitation: 495 nm, emission: 520 nm; see

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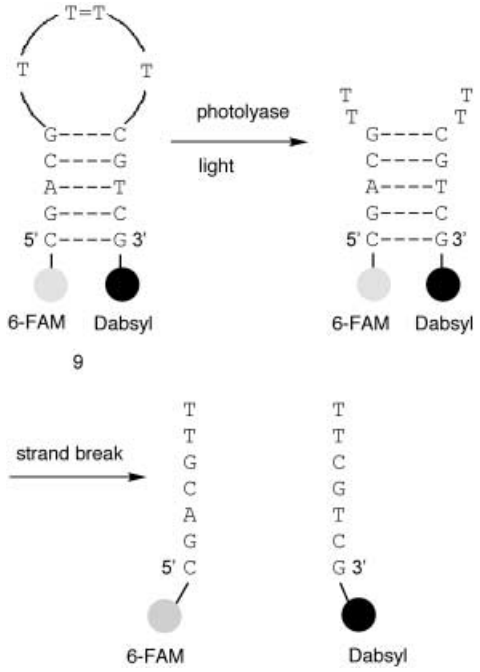
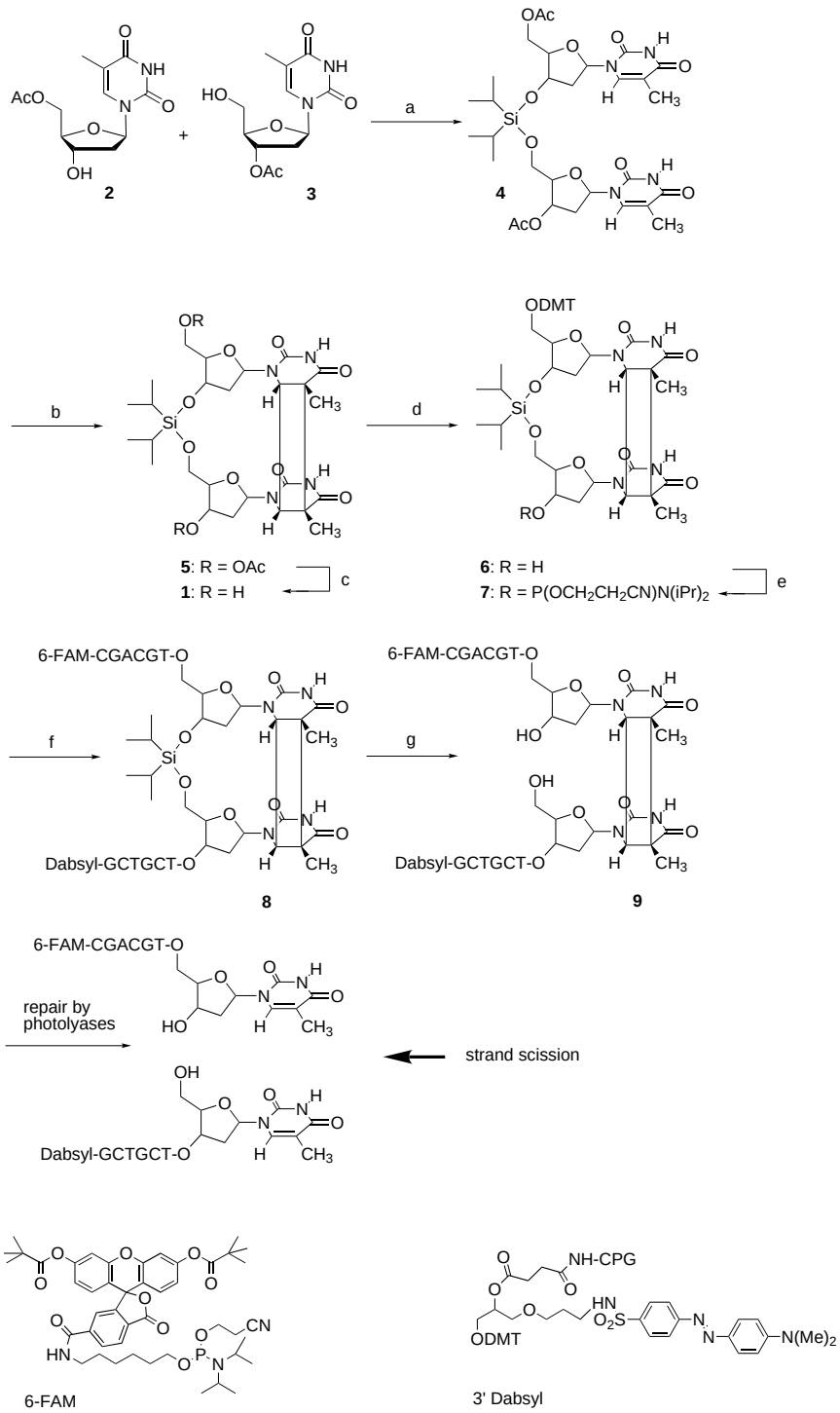


Figure 1. *Depiction of the molecular beacon strategy employed to quantify DNA repair activity. The beacon* ***9*** *contains a 5′-6-FAM fluorophore, a 3′-Dabsyl quencher, and the substrate analogue* ***1*** *in the loop of the hairpin.*

Scheme 1) and the quencher (Dabsyl), the molecular beacon contains a third chemical modification in a very short loop structure. This modification is a chemically synthesized substrate analogue, recognizable by the repair protein DNA photolyase (Scheme 1). The substrate analogue was designed to translate photolyase activity into a strand break. Hairpin cleavage then causes formation of a temporary double strand, which readily separates.

DNA photolyases were chosen because they are important enzymes for the repair of UV-induced carcinogenic[29] and antiproliferating[30] cyclobutane pyrimidine dimer lesions in the genomes of many organisms, and particularly in plants. Here they represent the major defense system against UV-induced cell death.[31, 32] DNA photolyases are light-activated enzymes that trigger an electron transfer from a reduced and deprotonated flavin coenzyme ($FADH^-$) to the thymine cyclobutane dimer.[33, 34] The reduced dimer undergoes a spontaneous cycloreversion, as depicted in Scheme 1. Photolyases are monomeric proteins with a molecular weight of about 55 kDa that contain—besides the $FADH^-$ electron donor—a second antenna pigment,[35] which is either an 8-hydroxy-5-deazaflavin[36, 37] or a methenyltetrahydrofolate.[38, 39] Photolyase repair proteins share with most other repair proteins the feature of low abundance in cells. In fact, A. Sancar and co-workers estimated that every photolyase-containing cell contains approximately 100 photolyase proteins.[31] Photolyases are divided into two classes (I and II).[40] Class I are the well studied microbial photolyases,[41, 42] while class II photolyases are present



Scheme 1. *Depiction of the fluorescein fluorophore 6-FAM and of the 3′-diazabenzylsulfonyl quencher Dabsyl, and the synthesis of the cleavable thymidine dimer substrate* ***1*** *and of the molecular beacon* ***9****: a) $(iPr)_2SiCl_2$, dimethylformamide (DMF), $-40\,^{\circ}C$, 79%; b) $h\nu > 300$ nm, 18 h, CH_3CN/H_2O, acetophenone 3.5 vol.%, 20%; c) NH_3 in dry MeOH; d) dimethoxytrityl chloride (DMTr-Cl), pyridine, molecular sieves (3 Å), 61% over steps c + d; e) $NCCH_2CH_2OP(Cl)N(iPr)_2$, dry tetrahydrofuran (THF),* N,N-diisopropylethylamine, 81%.

in higher organisms such as plants. These photolyases are not well understood, and their sequence homology with class I photolyases is small.[43] Fast (high-throughput screening) and reliable analysis is particularly desirable for class II photolyases[44, 45] because it may in the future allow direct quantification of the UV stress tolerance of higher organisms, particularly of crop plants.

2. Results and Discussion

Synthesis of the lesion substrate analogue and of the hairpin

Towards these goals, we performed the synthesis of a cleavable substrate analogue within a molecular beacon as depicted in Scheme 1. In analogy to an approach by J.-S. Taylor,[46] the natural substrate for the enzyme, the thymidine dimer **1**, was synthesized with a silyl diether bridge, which was cleaved later in the synthesis.[47, 48] This process allows photolyase repair activity to be translated into a strand break, as shown in Scheme 1. Photolyases accept this opened-backbone substrate analogue, most probably because they do not contact the central phosphodiester in the photolyase–DNA complex. This fact was established by A. Sancar by using data from detailed footprinting studies.[31]

The starting materials for the preparation of **1** are the 5′- and 3′-acetyl-protected thymidines **2**[49] and **3**.[50] The two building blocks were linked with diisopropylsilyl dichloride to give the dinucleotide **4**. Irradiation of **4** in a water/acetonitrile mixture containing acetophenone as the triplet sensitizer, in a Pyrex apparatus (cut-off $<$ 300 nm) and with a medium-pressure mercury lamp, provided the *cis-syn* thymidine dimer **5** in 20% yield, together with the *trans-syn* isomers (not shown; 40% yield).[51] Separation of the *cis-syn* dimer **5** was achieved by flash chromatography on silica-*H*. Cleavage of the undesired *trans-syn* byproducts back into **4** was accomplished by irradiation with 254 nm light. This method allowed the unwanted *trans-syn* isomer to be recycled, which increased the final reaction yield of the *cis-syn* compound **5** to about 30%. Cleavage of the acetyl protecting groups to give **1** was difficult because of concomitant hydrolysis of the silyl ether linkage. The silyl ether bridge in **5** shows greater lability than that in **4**, possibly as a result of steric strain. The cleavage of the two acetyl protecting groups to give the *cis-syn* thymidine dimer **1** was finally achieved with ammonia in dry methanol. Conversion of **1** into the phosphoramidite **7**, via **6**, was achieved by standard procedures. The synthesis of the molecular beacon **8**, 5′-d(6-FAM-CGACGTXTCGTCG-Dabsyl)-3′; (X = compound **1**) was performed by a standard phosphoramidite[52, 53] coupling procedure with commercially available reagents on a Dabsyl-containing solid support. The cleavage of the oligonucleotide from the solid support, of all base-protecting groups, and of the silyl ether linkage, was carried out in one step by treatment of the controlled-pore-glass (CPG)-supported oligonucleotide with an ammonium hydroxide/ethanol (3:1) solution at 55 °C for about 10 h. The final hairpin **9** was subsequently purified by reversed-phase HPLC (see the Experimental Section for details).

Characterization of the molecular beacon

The required sensitivity of the assay method led us to pay particular attention to the purity of the molecular beacon **9**. The purity of the synthesized photolyase-cleavable molecular beacon was investigated by reversed-phase HPLC, which showed a single, sharp peak at a retention time of about 20 min (Figure 2).

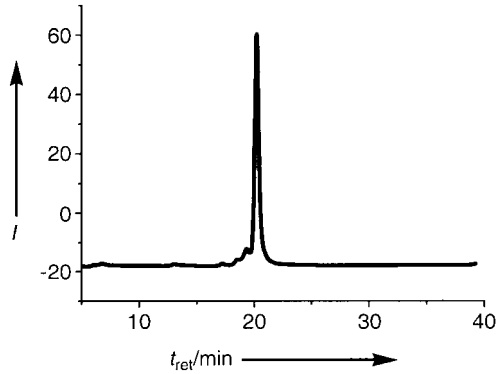


Figure 2. *HPLC trace of the molecular beacon* ***9****. Buffer: A = 0.1 M AcOH/NEt_3 in water, pH 7.0; B = 0.1 M AcOH/NEt_3 in 80% acetonitrile, pH 7.0; Gradient: 0–45% B over 35 min, 55 °C. Column: Nucleosil 250 mm × 4 mm , 100 Å, 3 μm.* t_{ret} *= retention time; I = relative fluorescence intensity.*

However, we had to perform the HPLC analysis at an elevated temperature (55 °C) with a RPC18 HPLC column with a particle size of 3 μm, presumably as a result of the strong self-pairing property of the designed molecular beacon, which forces the oligonucleotide into the U-conformation. Colder conditions or the use of the standard 5-Å column gave a broad and unresolved peak. The MALDI-TOF spectrum depicted in Figure 3 displays the expected molecular weight for the threefold modified molecular beacon **9** (**9**$^+$: MW = 5218), plus that of the potassium adduct ([**9** + K]$^+$: MW = 5257).

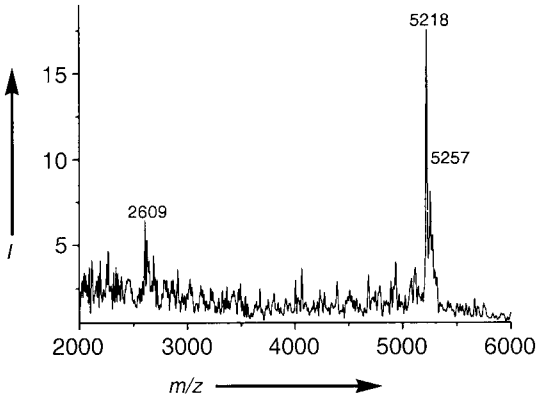


Figure 3. *MALDI-TOF mass spectrum of the molecular beacon* ***9****. Matrix: THA/citrate (2:1) in ethanol.*

In order to investigate the pairing and cleaving properties of the molecular beacon **9** and to study the fluorescence characteristics, we measured the fluorescence melting curve (Figure 4).[54] The determined melting point $T_m \approx 70$ °C shows that the hairpin

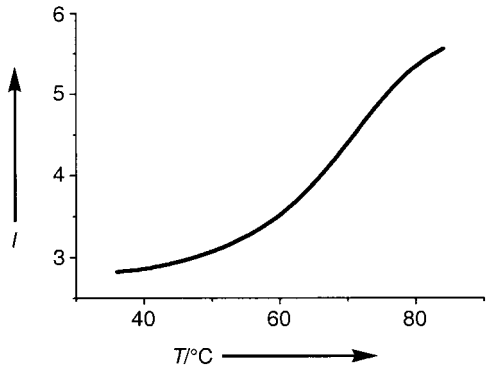


Figure 4. Fluorescence melting curve of the hairpin **9** *(3 μM DNA, 150 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5).* $T_m \approx 70\,^{\circ}C$. I = *relative fluorescence intensity.*

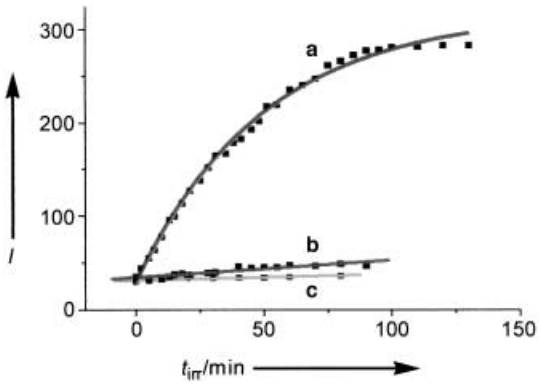


Figure 5. Fluorescence-based repair study with the A. nidulans *photolyase. Assay solution: molecular beacon* **9** *(0.2 μM), buffer (150 mM NaCl, 10 mM Tris, pH 7.5), 1,4-dithiothreitol (DTT; 10 mM), and* A. nidulans *photolyase (0.15 μM). Temperature: 27 °C. a) Assay solution irradiated with white light. b) Dark control. c) Assay solution containing no photolyase enzyme irradiated with white light.* t_{irr} = *irradiation time;* I = *relative fluorescence intensity.*

9 is certainly closed at room temperature and forms the U-type structure. Melting of the hairpin causes the fluorescence intensity to increase by a factor of two, which shows that the small size of the oligonucleotide still enables efficient energy transfer even in the stretched-out conformation.

Investigation of the duplex stability of the double strand (5′-CGACGTT-3′:3′-GCTGCTT-5′), which is the presumed product obtained after photolyase repair, gave no observable melting point, which shows that cleavage of the thymidine dimer substrate will lead to complete stem dissociation at room temperature (data not shown).

Photolyase assays performed with the cleavable substrate containing the molecular beacon

To address the question of whether repair of the thymidine dimer analogue in the loop of the molecular beacon allows quantification of photolyase activity, we studied the fluorescence increase in a fluorimeter with a thermostated cuvette. For the assay, 100 μL of a solution containing 0.2 μM **9** was prepared. The well-characterized class I photolyase from *Anacystis nidulans* photolyase (0.15 μM) was then added and the assay solution was continuously irradiated with white light at 27 °C. The irradiation was stopped from time to time and the fluorescence of the assay solution was monitored. The obtained increase in sample fluorescence is depicted in Figure 5 (curve a). It is clearly evident that the expected dimer repair gives rise to a strong fluorescence signal. The fluorescence intensity rises by a factor of more than 10. Curves b and c in Figure 5 show results from two control experiments performed to quantify the background activity. Curve b depicts the fluorescence increase of an identical assay solution kept in the dark, while curve c shows data obtained on irradiation of the hairpin **9** without any added photolyase.

To prove that the fluorescence increase (Figure 5, curve a) is in fact the result of hairpin cleavage at the dimer site, we also analyzed the assay solutions by HPLC. The experiment was performed as described above, but the small aliquots removed from the assay solution were then analyzed by HPLC with a fluorescence detector. The obtained chromatograms are depicted in Figure 6. At a retention time of about 20 min we observed a small peak caused by the hairpin **9**. Upon irradiation in the presence of *A. nidulans* photolyase, a second peak appeared at a retention time of approximately 10 min. Co-injection studies showed that the peak represented the 6-FAM-labeled single strand 5′-FAM-CGACGTT-3′, which is the repaired product. The much higher peak intensity of this peak in comparison to the original hairpin **9** again shows the disappearance of the energy transfer from the fluorescein to the Dabsyl quencher.

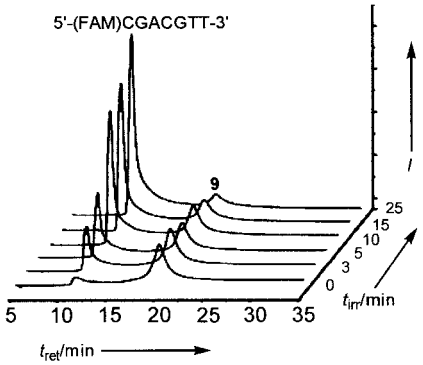


Figure 6. Series of HPLC traces of the assay solution irradiated with white light. Assay solution: molecular beacon **9** *(4 μM), buffer (150 mM NaCl, 10 mM Tris, pH 7.5), DTT (10 mM), and* A. nidulans *photolyase (0.15 μM). Temperature: 27 °C. HPLC conditions: Nucleosil column 250 × 4 mm, 100 Å, 3 μm; buffer: A = 0.1 M AcOH/*NEt_3 *in water, pH 7.0; B = 0.1 M AcOH/*NEt_3 *in 80% acetonitrile, pH 7.0; gradient: 0–45% B in 35 min. Temperature: 55 °C.* t_{ret} = *retention time;* t_{irr} = *irradiation time;* I = *relative fluorescence intensity.*

In order to investigate whether the molecular beacons allow analysis of repair activity even in crude cell extracts, particularly in plant cell extracts, we next analyzed whether the dimer analogue is accepted as a substrate by class II photolyases. Here we chose the enzyme (At-PHR1) from the plant *Arabidopsis thaliana*. The enzyme, which carried a C-terminal 6 × His tag, was expressed in *Escherischia coli* and affinity-purified on a Ni column.[55] The protein was isolated in a form that contained

the FAD cofactor but not second antenna pigment, which should strongly decrease the activity of the recombinant enzyme. Despite the low activity, the molecular beacon assay allowed rapid analysis of the *A. thaliana* photolyase activity as depicted in Figure 7 even without an excess of the hairpin. Curve a shows the rapid fluorescence increase of an assay solution (100 μL) containing 0.2 μM beacon **9** and 1.2 μM *A. thaliana* enzyme upon irradiation of the assay at 366 nm (energy fluence rate 44 W m^{-2}). These data prove that the molecular beacon assay allows rapid and reliable analysis even of class II enzymes.

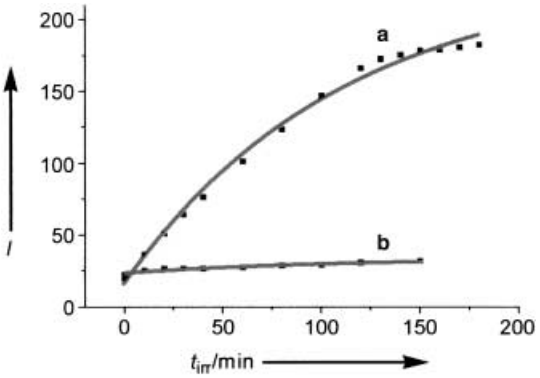


Figure 7. *Fluorescence-based repair study with the* A. thaliana *enzyme. Assay composition: molecular beacon* ***9*** *(0.2 μM), buffer (150 mM NaCl, 10 mM Tris, pH 7.5), DTT (10 mM), and* A. thaliana *enzyme (1.2 μM). Temperature: 27 °C. a) Assay solution irradiated at 366 nm (44 Wm^{-2}). b) Dark control.* t_{irr} *= irradiation time;* I = *relative fluorescence intensity.*

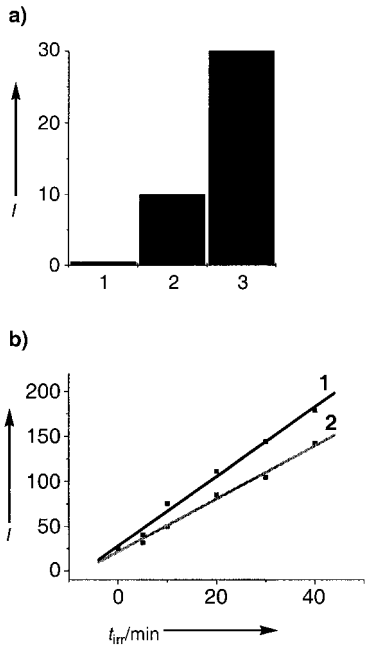


Figure 8. *Fluorescence-based repair study in cell extracts. Assay composition: molecular beacon* ***9*** *(0.2 μM), buffer (150 mM NaCl, 10 mM Tris, pH 7.5), DTT (10 mM). Total protein concentration: 0.7 μg per μL assay solution. Temperature: 27 °C. a) Bar graph representation of the assay data. Each bar shows the difference in fluorescence response between a sample kept in the dark and a sample irradiated at 366 nm (44 Wm^{-2}) for 60 min: 1) an assay containing just the molecular beacon, 2) an assay containing protein extract from the photolyase mutant UVR2—1, 3) an assay containing protein extract from wild-type plants. b) Time-dependent increase of the fluorescence response from a sample containing the wild-type cell extract and the molecular beacon* ***9****. 1: Irradiated assay solution with wild-type extract. 2: Assay solution kept in the dark.* t_{irr} = *irradiation time;* I = *relative fluorescence intensity.*

Activity profiling of class II photolyase in crude cell extracts

With a sensitive assay for class II photolyases to hand, we next turned to the analysis of cell extracts (Figure 8 a, bar graph 3) from wild-type *A. thaliana* plants. As a control, cell extracts were also prepared from *A. thaliana* plants lacking the *PHR1* photolyase gene[56] (*phr1* mutant, bar graph 2). We first studied the stability of the molecular beacon in the crude cell extracts. To this end, hairpin **9** was added to a cell extract solution, which was stirred overnight under strict exclusion of light. Analysis of the solution by HPLC revealed approximately 50 % degradation over this time period, which defines the background reaction. To our surprise we observed no degradation of the hairpin into small fragments but cleavage of the hairpin at the dimer site. This cleavage could potentially result from an unknown DNA repair enzyme activity (for example, a glycosylase activity) that operates in the dark. It may, alternatively, reflect a particular vulnerability of the dimer-containing loop towards nuclease digestion. Further experiments to investigate this question are currently in progress.

In order to study the possibility of detecting photolyase activity in these cell extracts, we prepared another 100-μL assay solution, which contained 0.2 μM hairpin **9** and a cell extract solution with 0.7 μg total protein per μL (70 μg total protein extract in the assay solution). These assay solutions were irradiated for just 1 h at room temperature at $\lambda = 366$ nm (44 W m^{-2}). The background level was determined from an identical reference assay solution that contained the same amount of protein extract and was kept in the dark.

Relative to the mutant control depicted in Figure 8 a, bar 2, in which we also plotted the difference in the fluorescence response between the irradiated sample (366 nm, 44 W m^{-2}) and the dark control, a reproducible threefold increase in fluorescence signal was observed in the assay solution that contained the wild-type cell extract (Figure 8 a, bar 3). This result indicates reduced photolyase activity in the mutant plant. Bar 1 (Figure 8 a) shows an experiment identical to that above but with an assay containing only the molecular beacon and no extract, which shows almost no change in fluorescence response.

We had expected zero photolyase activity in the mutant plants and wondered why this experiment still gave a light-induced cyclobutane pyrimidine dimer (CPD) repair response. Control experiments in which the cell extracts were incubated with the hairpin **9** and the cleavage products were analyzed by gel electrophoresis confirmed, however, that the mutant *A. thaliana* plants still possess some, though reduced, CPD-splitting activity. The activity is clearly above the background level and is light dependent, which excludes the possibility that the data are a result of the action of any nucleases. We can conclude from our

experiments that the extract experiments are very difficult. We observe some cleavage activity of the beacon **9** at the dimer site in the dark. On top of this background activity we clearly see an additional light-induced response. This response is demonstrated in Figure 8b, which shows the time-dependent fluorescence increase. All obtained data were highly reproducible and similar data were obtained with different extracts prepared from different plants. The results demonstrate that the new beacon assay is in fact able to measure class II photolyase activities reliably even in crude protein extracts. Nevertheless, the cell extract studies are clearly at the limit of what can be measured with the hairpin **9**. In order to investigate the detection limit further, we added a small amount of *A. nidulans* photolyase during a cell extract assay and measured the repair rate increase. A systematic study showed that 5 – 10 pmol photolyase is clearly detectable with the hairpin method.

3. Conclusion

The development of new analytical methods that allow quick determination of protein activities inside and outside cells is desirable for proteomics research. A novel molecular beacon **9**, which contains a chemically synthesized substrate analogue in the head region, was prepared for real-time monitoring of enzyme activity. The substrate analogue allows photolyase activity to be translated into hairpin cleavage, which gives rise to a strong fluorescence signal. The designed molecular beacon was found to allow rapid activity profiling of purified class I and class II photolyases. Most importantly, real-time monitoring of photolyase activity even in crude cell extracts was possible, albeit at the sensitivity limit. The new assay is therefore suitable for quantification of the UV stress tolerance of plants.

Experimental Section

General: Melting points are uncorrected. ^{1}H NMR spectra were recorded on Varian Gemini 200 (200 MHz) and 300 (300 MHz) and Bruker AMX 300 (300 MHz), 400 (400 MHz), and 500 (500 MHz) spectrometers. The chemical shifts were referenced to $CHCl_3$ ($\delta =$ 7.27 ppm) in $CDCl_3$ and DMSO ($\delta = 2.50$ ppm) in DMSO-d_6. ^{13}C NMR spectra were recorded on Bruker DRX 200 (50 MHz) and AMX 500 (125 MHz) spectrometers, and the chemical shifts were referenced to $CHCl_3$ in $CDCl_3$ and DMSO in DMSO-d_6. Standard pulse sequences were employed for ^{1}H 2D NOESY and ^{1}H,^{1}H correlation studies. IR spectra were recorded in KBr and measured with a Bruker IFS 25 Fourier transform infrared spectrophotometer. Mass spectra and high-resolution mass spectra were measured on VG ZAB2-SEQ (nitrobenzyl alcohol matrix), Finnigan TSQ 700, Finnigan MAT 95S, Bruker Biflex (MALDI-Tof), and HP5989B mass spectrometers. HPLC was performed with a Merck – Hitachi system equipped with L-7400 UV and L-7480 fluorescence detectors. Analytical separations were performed with a Machery – Nagel Nucleosil 100 – 3 C18 (250 × 4 mm) column. A Nucleoprep 100 – 12 C18 column (250 × 40 mm) was used for preparative separations.

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.

Synthesis of oligonucleotides and of the molecular beacon 9: The synthesis of the molecular beacon was performed by a standard phosphoramidite procedure with commercially available phosphoramidites (Pharmacia) on an Expedite 8900 DNA synthesizer. The coupling efficiency was monitored with a trityl monitor. The cleavage of the oligonucleotide from the solid support and the cleavage of all protecting groups and of the silyl spacer was carried out in one step by shaking the CPG-supported oligonucleotide in an ammonia/ethanol (3:1) solution for about 10 h at 55 °C in a thermostated container. All oligonucleotides were purified by reversed-phase HPLC (RPC-18, 0 – 50% acetonitrile in a 0.1 M aqueous solution of ammonium bicarbonate, followed by exhaustive coevaporation with water/ethanol). The oligonucleotides were characterized by matrix-assisted laser desorption/ionization mass spectrometry. A stock solution of the molecular beacon was prepared in water.

Repair studies with the purified *A. nidulans* photolyase: Repair measurements were performed with a fluorescence spectrometer (JASCO-FP-750). An assay solution (100 μL) containing molecular beacon **9** (0.2 μM), photoreactivation buffer (NaCl, 150 mM; Tris, 10 mM), DTT (10 mM), and *A. nidulans* enzyme (0.15 μM) was prepared in a fluorescence cuvette. The assay solution was irradiated with white light and the fluorescence intensity was measured from time to time. The experiment was performed at 27 °C. The dark control was carried out simultaneously in a similar cuvette and under identical conditions. Irradiation in the absence of photolyase was also performed under similar conditions. Analysis of the repair reaction by HPLC was performed with an assay solution (200 μL) containing molecular beacon **9** (4 μM), buffer (NaCl, 150 mM; Tris, 10 mM; pH 7.5), DTT (10 mM), and *A. nidulans* photolyase (0.2 μM). The solution was again irradiated with white light. Samples (10 μL) were taken after defined time intervals. An acetic acid (20%, 20 μL) solution was added to these samples to stop any further repair. HPLC analysis was performed under the following conditions: Nucleosil RP-C18 column; A = 0.1 N AcOH/NEt_3 in water, pH 7.0; B = 0.1 N AcOH/NEt_3 in 80% acetonitrile, pH 7.0; 0 – 45% B over 35 min.

Repair studies with the purified *A. thaliana* photolyase: The repair experiment was carried out as described above. The assay solution (100 μL) contained the molecular beacon **9** (0.2 μM), photoreactivation buffer (see above), and *A. thaliana* photolyase at a concentration of 1.2 μM. All irradiation was carried out with a 366 nm lamp (energy fluence rate 44 W m^{-2}) at 27 °C. The dark control was performed simultaneously, but the assay solution was kept in the dark. Total protein extracts were prepared from rosette leaves of 21 day-old *A. thaliana* wild-type (ecotype *Landsberg erecta*) and photolyase mutant *uvr2 – 1*[56] plants essentially as described previously.[55]

Repair study with cell extracts: Repair experiments were carried out as described above. The assay solution (100 μL) contained the molecular beacon **9** (0.2 μM), photoreactivation buffer (see above), and cell extracts at a final concentration of 0.7 μg μL^{-1}. All irradiation was carried out with a 366 nm lamp (44 W m^{-2}) at 27 °C. As a background control, another assay solution was kept in the dark.

5′-*O*-Acetyl-thymidyl-3′,5′-*O*-diisopropylsilyl-3′-*O*-acetyl-thymidine (**4**): 2,6-Di-*tert*-butyl-4-methylpyridine (2.7 g, 13.00 mmol) was dissolved in MeCN (35 mL). Diisopropylsilyl bis(trifluoromethanesulfonate) was added (3.5 mL, 11.9 mmol). The solution was cooled to −40 °C. Subsequently, a solution of 5′-acetylthymidine **2**[49] (3.12 g, 11.0 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (600 mg, 2.9 mmol) in DMF (8 mL) was added dropwise over half an hour. The reaction mixture was stirred for another half an hour at −40 °C. After addition of 3′-acetylthymidine (**3**,[50] 3.04 g, 10.7 mmol) in DMF (5 mL) the reaction mixture was stirred for 1 h at RT. The mixture was poured into ice-cold water (4 L) and the precipitate was filtered off.

Product **4** was purified by flash chromatography ($CHCl_3$/MeOH 40:1) and isolated in the form of a colorless powder (5.7 g, 8.4 mmol, 79% based on 3′-acetylthymidine (**3**). $R_f = 0.26$ ($CHCl_3$/MeOH 10:1); m.p.: 76–78 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.00–1.09$ (m, 14H; $SiC(CH(CH_3)_2)_2$), 1.89 (s, 3H; C5CH_3), 1.91 (s, 3H; C5CH_3), 2.08 (s, 3H; H_3COO), 2.09 (s, 3H; H_3COO), 2.10–2.18 (m, 2H; CA2′H_2), 2.38–2.45 (m, 2H; CB2′H_2), 3.96–4.03 (m, 2H), 4.06–4.11 (m, 2H), 4.16–4.18 (m, 1H), 4.27–4.29 (m, 2H), 4.52–4.56 (m, 1H), 6.24–6.35 (m, 2H, CA1′H, CB1′H), 7.26 (s, 1H; HC6), 7.36 (s, 1H; HC6), 9.55 (s, 1H; NH), 9.61 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 11.87$, 12.50 (2C), 12.63 (2C), 12.68, 17.35 (2CSi), 20.89, 21.02, 37.69, 40.82, 72.53, 72.64, 74.10, 74.34, 84.44, 84.61, 84.95, 85.50, 111.15, 111.39, 134.68, 135.04, 150.36, 150.56, 163.85, 163.98, 170.34, 170.66 ppm; IR (KBr): 3200 (w), 3067 (w), 2945 (m), 1745 (s), 1694 (s), 1467 (m), 1367 (m), 1272 (m), 1239 (s), 1200 (m), 1128 (m), 1072 (m), 956 (w), 883 (m), 811 (w), 778 (w), 695 (w), 611 (w), 556 (w), 483 (w), 417 (w) cm^{-1}; MS (positive FAB): 1361.8 (28, $[2 \times M+H]^+$), 703.1 (15, $[M+Na]^+$, 682.2 (27), 681.2 (66, $[M+H]^+$); HR-MS (positive FAB) calcd for $[C_{30}H_{44}N_4O_{12}Si+H]^+$: 681.2803; found: 681.2805.

5′-*O*-Acetyl,3′-*O*-acetyl-5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*)-[*cis,syn*]-cyclobutane thymidine dimer 5: General method: Compound **4** (about 1 g) was dissolved in H_2O/MeCN (350 mL, 1:1). Acetophenone (1 mL) was added, and the solution was degassed by bubbling N_2 through the solution for 0.5 h. The solution was subsequently irradiated for 18 h with a 150 W (TQ-150) medium-pressure mercury lamp in a Pyrex irradiation apparatus with a cut-off at 300 nm. MeCN was removed under a vacuum. The aqueous phase was saturated with NaCl and extracted with $CHCl_3$ (10 times, each with 150 mL). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated in a vacuum. The *cis-syn* product **5** was isolated by flash chromatography ($CHCl_3$/MeOH 50:1) and obtained in the form of a colorless powder. Irradiation of 5.5 g of **4** in five portions provided 1.2 g (1.7 mmol, 21%) of the *cis-syn* dimer **5**. The *trans-syn* dimers were eluted together and were obtained in about 40% yield [$R_f =$ 0.59 ($CHCl_3$/MeOH 10:1)]. This material was split back into **4** by irradiation of a solution containing these isomers in H_2O/MeCN (1:1) under anaerobic conditions for 0.5 h with a 150-W (TQ-150) medium-pressure mercury lamp in a quartz irradiation apparatus (254 nm). *cis-syn* Dimer **5**: $R_f = 0.27$ ($CHCl_3$/MeOH 10:1); m.p.: 145–148 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.93–1.08$ (m, 14H; $SiC(CH(CH_3)_2)_2$), 1.42 (s, 3H; C5CH_3), 1.50 (s, 3H; C5CH_3), 2.06 (s, 3H; H_3COO), 2.07 (s, 3H; H_3COO), 2.15–2.35 (m, 3H; CA2′H_2, CB2′H_{2a}), 2.70–2.78 (m, 1H; CB2′H_{2b}), 3.86–3.96 (m, 4H), 4.06–4.30 (m, 5H), 4.88–4.93 (m, 1H; C(3′)H), 5.91–6.01 (m, 2H; CA1′H, CB1′H), 9.40 (s, 1H; NH), 9.57 (s, 1H; NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.11$, 12.26, 16.99, 17.08, 17.32 (2C), 17.40, 17.84, 18.22, 20.91, 37.10, 38.15, 47.44, 50.51, 56.84, 57.47, 63.55, 63.63, 71.09, 77.29, 81.53, 83.27, 85.13, 85.21, 152.32, 152.43, 168.85, 170.58, 170.71, 171.79 ppm; IR (KBr): 3433 (m), 3244 (m), 3089 (w), 2944 (m), 2856 (m), 1711 (s), 1461 (m), 1383 (m), 1272 (m), 1239 (s), 1094 (m), 1056 (m), 878 (w), 794 (w), 756 (w), 694 (w) cm^{-1}; MS (positive FAB): 1361.4 (9, $[2M+H]^+$), 681.3 (38, $[M+H]^+$), 621.3 (59, $[M - CH_3COO]^+$), 523.3 (100); HR-MS (positive FAB) calcd for $[C_{30}H_{44}N_4O_{12}Si+H]^+$: 681.2803; found: 681.2806.

5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*)-[*cis,syn*]-cyclobutane thymidine dimer 1: Compound **5** (1.05 g, 1.55 mmol) was dissolved at 0 °C in dry methanol saturated with NH_3 (100 mL) and stirred for 6 h. The solvent was removed in vacuum at RT. The obtained product was not purified but directly introduced into the next step. $R_f = 0.34$ ($CHCl_3$/MeOH 5:1); m.p.: 151–153 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 0.99–1.18$ (m, 14H; $((CH_3)_2CH)_2Si$), 1.42 (s, 3H; C5CH_3), 1.44 (s, 3H; C5CH_3), 2.02–2.18 (m, 4H; C(A2′)H_2, C(B2′)H_2), 2.85–2.97 (m, 1H), 2.62–2.81 (m, 5H), 3.94–4.99 (m, 6H), 4.44–4.47 (m, 1H), 4.56–4.63 (m, 1H), 5.91–6.04 (m, 2H; C(A1′)H, C(B1′)H) ppm; ^{13}C NMR (75 MHz, CD_3OD): $\delta = 12.79$, 12.97, 13.02, 13.12, 18.44, 18.56, 18.63, 18.67, 23.76, 37.68, 47.27, 51.06, 55.61, 58.37, 61.12, 65.76, 69.81, 71.28, 85.44, 85.87, 86.41, 86.55, 153.61, 154.65, 171.29, 172.61; IR (KBr): 3444 (m), 3089 (w), 2944 (m), 2867 (m), 1700 (s), 1461 (m), 1389 (m), 1283 (m), 1094 (s), 1050 (m), 883 (w), 794 (w), 756 (w), 694 (w) cm^{-1}; MS (positive FAB): 1215.5 (10, $[2M+Na]^+$), 619.3 (100, $[M+Na]^+$); HR-MS (positive FAB): calcd for $[C_{26}H_{40}N_4O_{10}Si+Na]^+$: 619.2411; found: 619.2407.

5′-*O*-Dimethoxytrityl-5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*)-[*cis,syn*]-cyclobutane pyrimidine dimer 6: The *cis-syn* compound **1** (680 mg) was dissolved in pyridine (2 mL) and the solvent was then removed in a vacuum under argon. This procedure was repeated five times. The material was again dissolved in dry pyridine (3 mL) and molecular sieves (4 Å) were added. This mixture was stirred for 3.5 h at RT. Dimethoxytrityl chloride (596 mg, 1.1 mmol, 1.1 equiv.) was then added and the reaction mixture was stirred until the reaction was complete. After addition of MeOH (1 mL), the solvent was removed in a vacuum at RT. The product **6** was purified by flash chromatography ($CHCl_3$/MeOH/Py 10:1:0.1) to give compound **6** in the form of a colorless powder (626 mg, 0.70 mmol, 61%). $R_f = 0.48$ ($CHCl_3$/MeOH 10:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.72–1.04$ (m, 20H; $2 \times H_3CC(5)$), $((H_3C)_2CH)_2Si$), 1.92–2.02 (m, 1H; $H_2C(2')$), 2.20–2.29 (m, 1H; $H_2C(2')$), 2.36–2.42 (m, 1H; $H_2C(2')$), 2.75 (m, 1H; $H_2C(2')$), 3.17–3.21 (m, 1H), 3.62–3.65 (m, 1H), 3.78 (s, 3H; H_3CO), 3.79 (s, 3H; H_3CO), 3.82–3.88 (m, 4H), 3.94–4.00 (m, 1H), 4.08–4.14 (m, 1H), 4.21–4.24 (m, 1H), 4.31–4.33 (m, 1H), 4.60–4.62 (m, 1H), 5.96–6.00 (m, 1H; HC(1′)), 6.11–6.14 (m, 1H; HC(1′)), 6.80–6.83 (m, 4H; Ar), 7.22–7.42 (m, 9H; Ar), 9.09 (s, 1H; NH), 9.14 (s, 1H; NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 11.32$, 12.21, 12.43, 17.11, 17.25, 38.80, 40.32, 46.05, 48.23, 50.14, 55.25, 55.71, 56.84, 62.00, 64.43, 69.54, 69.68, 83.58, 84.74, 85.39, 85.83, 86.18, 113.04, 127.21, 127.79, 128.60, 130.41, 135.30, 135.49, 144.36, 152.53, 153.17, 158.72, 170.43, 171.44 ppm; IR (KBr): 3444 (w), 3222 (w), 3067 (w), 2944 (m), 2867 (m), 1705 (s), 1606 (m), 1506 (m), 1456 (s), 1389 (m), 1367 (m), 1289 (m), 1244 (s), 1178 (m), 1094 (m), 1028 (m), 961 (w), 878 (w), 828 (m), 789 (w), 750 (m), 700 (m), 583 (w) cm^{-1}; MS (positive FAB): 898.0 (13, $[M]^+$), 303.1 (100, $DMTr^+$); HR-MS (positive FAB): calcd for $[C_{47}H_{58}N_4O_{12}Si]^+$: 898.3821; found: 898.3828.

***cis-syn* Cyclobutane thymidine dimer phosphoramidite 7**: The dimethoxytrityl-protected dimer **6** (250 mg, 0.278 mmol) was dissolved in dry THF and the solvent was removed in a vacuum ($3 \times$ 2 mL). The material was again dissolved in dry THF (2 mL) under argon. Diisopropylethylamine (0.2 mL, 4 equiv) was then added. 2-Cyanoethoxy-(*N,N*-diisopropylamino)chlorophosphine (0.1 mL, 1.7 equiv) was added and the mixture was stirred for 1.5 h under argon at RT. The precipitate was filtered off. The solvent was removed in vacuum and the product was purified by flash chromatography ($CHCl_3$/MeOH/Py 10:1:0.1). Compound **7** was finally dissolved in CH_2Cl_2 (1 mL) and precipitated by addition of pentane (250 mL). The phosphoramidite **7** was obtained in the form of a colorless powder (248 mg, 0.22 mmol, 81%). $R_f = 0.65$ ($CHCl_3$/MeOH 10:1); m.p.: 146–149 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.89–1.29$ (m, 32H; $2 \times$ CHSi, $4 \times CH_3$CHSi, $4 \times CH_3$CHN, $2 \times CH_3$C5); 1.99–2.07 (m, 1H; C2B′H_{2b}), 2.19–2.27 (m, 1H; C2A′H_{2b}), 2.37–2.43 (m, 1H; C2B′H_{2a}), 2.60–2.68 (m, 2H; CH_2CN), 2.74–2.82 (m, 1H; C2A′$_{2a}$), 3.16–3.20 (m, 1H; C5H), 3.47–3.57 (m, 3H; $2 \times$ CHN, C5H), 3.63–3.96 (m, 12H; $2 \times OCH_3$ (s, 6H at 3.72), OCH_2CH_2, CA4′H, CB4′H, C(5′)H_2), 4.18–4.26 (m, 3H; CB3′H, C(5′)H), 4.59–4.64 (m, 1H; CA3′H)6.08 (*d*, ^3J(CB1′H,CB2′H) = 6.8 Hz, 1H; CB1′H), 6.11–6.23 (m, 1H; CA1′H), 6.82 (*d*, $^3J(C_{Ar}H,C_{Ar}H) =$ 8.4 Hz, 4H; $4 \times C_{Ar}H$), 7.25–7.29 (m, 7H; $C_{Ar}H$), 7.40 (*d*, $^3J(C_{Ar}H,C_{Ar}H) =$ 6.85 Hz, 2H; $2 \times C_{Ar}H$), 8.5972 (s, 2H; $2 \times$ NH ppm); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.27$, 12.35, 12.40, 12.46, 17.31, 20.08, 20.21, 20.34, 20.48, 20.62, 22.92, 22.98, 23.02, 23.06, 24.51, 24.64, 24.76, 38.42, 38.79, 38.93, 43.24, 43.49, 45.33, 45.45, 48.98, 49.20, 49.31, 49.59, 55.32,

56.54, 57.87, 58.00, 58.24, 58.36, 62.29, 63.54, 63.90, 70.03, 70.15, 70.63, 70.93, 71.31, 83.63, 84.39, 84.51, 84.64, 84.82, 84.93, 86.23, 113.15, 117.03, 117.61, 117.87, 127.22, 127.85, 128.59, 130.44, 135.45, 135.60, 144.54, 152.11, 152.27, 153.33, 153.42, 158.79, 170.39, 170.60, 170.87 ppm; ^{31}P NMR (121 MHz, $CDCl_3$): $\delta = 148.87$, 150.01 ppm; IR (KBr): 578 (w), 700 (m), 756 (m), 789 (m), 828 (m), 883 (m), 972 (m), 1033 (s), 1094 (m), 1178 (s), 1250 (s), 1367 (m), 1389 (m), 1456 (m), 1511 (m), 1606 (m), 1711 (s), 2867 (m), 2956 (m), 3078 (w), 3078 (w), 3233 (w), 3411 (w) cm^{-1}; MS (positive FAB): 1121.2 (13, $[M+Na]^+$), 1099.3 (18), 1098.2 (17, $[M]^+$), 303.1 (100, $DMTr^+$); HR-MS (positive FAB): calcd for $[C_{56}H_{75}N_6O_{13}Psi+H]^+$: 1099.4979; found: 1099.4981.

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[1] M. Schena, R. A. Heller, T. P. Theriault, K. Konrad, E. Lachmeier, R. W. Davis, *Trends Biotechnol.* **1998**, *16*, 301 – 306.
[2] D. J. Lockhart, E. A. Winzler, *Nature* **2000**, *405*, 827 – 836.
[3] N. L. Anderson, N. G. Anderson, *Electrophoresis* **1998**, *19*, 1853 – 1861.
[4] A. Pandey, M. Mann, *Nature* **2000**, *405*, 837 – 846.
[5] D. Figeys, A. Ducret, J. R. Yates III, R. Aebersold, *Nat. Biotechnol.* **1996**, *14*, 1579 – 1583.
[6] Y. Liu, M. P. Patricelli, B. F. Cravatt, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14694 – 14699.
[7] G. C. Adam, B. F. Cravatt, E. J. Sørensen, *Chem. Biol.* **2001**, *8*, 81 – 95.
[8] N. Winssinger, J. L. Harris, B. J. Backes, P. G. Schultz, *Angew. Chem.* **2001**, *113*, 3254 – 3258; *Angew. Chem. Int. Ed.* **2001**, *40*, 3152 – 3155.
[9] X. Fang, J.-J. Li, J. Perlete, W. Tan, K. Wang, *Anal. Chem.* **2000**, 747A – 753A.
[10] T. Förster, *Discuss. Faraday Soc.* **1959**, *27*, 7 – 17.
[11] S. Tyagi, F. R. Kramer, *Nature Biotech.* **1996**, *14*, 303 – 308.
[12] X. Fang, X. Liu, S. Schuster, W. Tan, *J. Am. Chem. Soc.* **1999**, *121*, 2921 – 2922.
[13] S. L. Beaucage, *Curr. Med. Chem.* **2001**, *8*, 1213 – 1244.
[14] J. J. Li, X. Fang, S. M. Schuster, W. Tan, *Angew. Chem.* **2000**, *112*, 1091 – 1094; *Angew. Chem. Int. Ed.* **2000**, *39*, 1049—1052.
[15] R. Bar-Ziv, A. Libchaber, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9068 – 9073.
[16] N. Hamaguchi, A. Ellington, M. Stanton, *Anal. Biochem.* **2001**, *294*, 126 – 131.
[17] S. Hashimoto, B. Wang, S. M. Hecht, *J. Am. Chem. Soc.* **2001**, *123*, 7437 – 7438.
[18] J. B. Biggins, J. R. Prudent, D. J. Marshall, M. Ruppen, J. S. Thorson, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13537 – 13542.
[19] J. J. Li, R. Geyer, W. Tan, *Nucleic Acids Res.* **2000**, *28*, 51 – 55.
[20] H. Schöneborn, J. Bülle, G. von Kiederowski, *ChemBioChem* **2001**, *2*, 922 – 927.
[21] K. E. Pierce, J. E. Rice, J. A. Sanchez, C. Brenner, L. J. Wangh, *Mol. Hum. Reprod.* **2000**, *6*, 1155 – 1164.
[22] B. Vogelstein, K. W. Kinzler, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9236 – 9241.
[23] S. Tyagi, S. A. E. Marras, F. R. Kramer, *Nat. Biotechnol.* **2000**, *18*, 1191 – 1196.
[24] J. A. M. Vet, A. R. Majithia, S. A. E. Marras, S. Tyagi, S. Dube, B. J. Poiesz, F. R. Kramer, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6394 – 6639.
[25] C. Reinbold, F. E. Gidow, E. Herrbach, V. Ziegler-Graff, M. C. Goncalves, J. F. J. M. van den Heuvel, V. Brault, *J. Gen. Virol.* **2001**, *82*, 1995 – 2007.
[26] C. Molenaar, S. A. Marras, J. C. M. Slats, J.-C. Truffert, M. Lemaître, A. K. Raap, R. W. Dirks, H. J. Tanke, *Nucleic Acids Res.* **2001**, *29*, 81 – 89.
[27] A. Tsuji, H. Koshimoto, Y. Sato, M. Hirano, Y. Sei-lid, S. Kondo, K. Ishibashi, *Biophys. J.* **2000**, *78*, 3260 – 3274.
[28] C.-H. Tung, U. Mahmood, S. Bredow, R. Weissleder, *Cancer Res.* **2000**, *60*, 4953 – 4958.
[29] J.-S. Taylor, S. Nadji, *Tetrahedron* **1991**, *47*, 2579 – 2590.
[30] J.-S. Taylor, *Acc. Chem. Res.* **1994**, *27*, 76 – 82.
[31] A. Sancar, *Biochemistry* **1994**, *33*, 2 – 9.
[32] T. Carell, L. T. Burgdorf, L. M. Kundu, M. K. Cichon, *Curr. Opin. Chem. Biol.* **2001**, 491 – 498.
[33] P. F. Heelis, R. F. Hartman, S. D. Rose, *Chem. Soc. Rev.* **1995**, 289 – 297.
[34] T. Carell, R. Epple, *Eur. J. Org. Chem.* **1998**, 1245 – 1258.
[35] M. S. Jorns, E. T. Baldwin, G. B. Sancar, A. Sancar, *J. Biol. Chem.* **1987**, *262*, 486 – 491.
[36] K. Malhotra, S.-T. Kim, C. Walsh, A. Sancar, *J. Biol. Chem.* **1992**, *267*, 15406 – 15411.
[37] S.-T. Kim, P. F. Heelis, A. Sancar, *Biochemistry* **1992**, *31*, 11244 – 11248.
[38] G. Payne, A. Sancar, *Biochemistry* **1990**, *29*, 7715 – 7727.
[39] S.-T. Kim, P. F. Heelis, T. Okamura, Y. Hirata, N. Mataga, A. Sancar, *Biochemistry* **1991**, *30*, 11262 – 11270.
[40] A. Yasui, A. P. M. Eker, S. Yasuhira, H. Yajima, T. Kobayashi, M. Takao, A. Oikawa, *EMBO J.* **1994**, *13*, 6143 – 6151.
[41] H.-W. Park, S.-T. Kim, A. Sancar, J. Deisenhofer, *Science* **1995**, *268*, 1866 – 1872.
[42] T. Tamada, K. Kitadokoro, Y. Higuchi, K. Inaka, A. Yasui, P. E. de Ruiter, A. P. M. Eker, K. Miki, *Nat. Struct. Biol.* **1997**, *11*, 887 – 891.
[43] S. Kanai, R. Kikuna, H. Toh, H. Ryo, T. Todo, *J. Mol. Evol.* **1997**, *45*, 535 – 548.
[44] M. S. Jorns, *Biochemistry* **1985**, *24*, 1856 – 1861.
[45] M. A. Smith, *Can. J. Zool.* **2000**, *78*, 1869 – 1872.
[46] S. Nadji, C.-I. Wang, J.-S. Taylor, *J. Am. Chem. Soc.* **1992**, *114*, 9266 – 9269.
[47] R. J. Lewis, P. C. Hanawalt, *Nature* **1982**, *298*, 393 – 396.
[48] D. A. Vivic, D. T. Odom, M. E. Nunez, D. A. Gianolio, L. W. McLaughlin, J. K. Barton, *J. Am. Chem. Soc.* **2000**, *122*, 8603 – 8611.
[49] A. M. Michelson, A. R. Todd, *J. Am. Chem. Soc.* **1953**, *75*, 951 – 959.
[50] G.-X. Hee, N. Bischofberger, *Nucleosides Nucleotides* **1997**, *16*, 257 – 263.
[51] J. Butenandt, A. P. M. Eker, T. Carell, *Chem. Eur. J.* **1998**, *4*, 642 – 653.
[52] L. J. McBride, M. H. Caruthers, *Tetrahedron Lett.* **1983**, *24*, 245 – 248.
[53] N. D. Sinha, J. McManus, H. Köster, *Nucleic Acids Res.* **1984**, *12*, 4539 – 4557.
[54] K. J. Breslauer, *Methods Enzymol.* **1987**, *259*, 221 – 242.
[55] O. Kleiner, J. Butenandt, T. Carell, A. Batschauer, *Eur. J. Biochem.* **1999**, *264*, 161 – 167.
[56] L. G. Landry, A. E. Stapleton, J. Lim, P. Hoffman, J. B. Hays, U. Walbot, R. L. Last, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 328 – 332.

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