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Light-Induced Formation of G-Quadruplex DNA Secondary Structures

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The attachment of photolabile "protecting" groups to mask the activity of biologically active compounds is commonly referred to as "caging".[1] The active molecule can be released by irradiation with the laser of a confocal microscope. The method provides exact control over the location, dose and time at which this event occurs. The strengths of this approach lie, for example, in the arbitrary choice of location where a pro-

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cess can be triggered in a suitable (light-accessible) model organism[2] and in the possibility of studying kinetic events after initiation by a light pulse.[3]

Recently, we have started to prepare DNA and RNA derivatives that bear caging groups on their nucleobases, which prevent them from forming Watson–Crick base pairs. In contrast to other groups that have chosen to mainly cage backbone phosphate groups of DNA[4] and RNA,[2,5] we want to introduce the modifications at specific sites[6]—ideally in any given sequence—and produce clean products of established identity for a better on/off behaviour. This is because it turns out that not all caged modifications are inactive and not all modifications in different positions can be removed with equal ease. To this end, we first prepared a caged analogue of thymidine (T^{NPP}) that contained a photolabile 2-(2-nitrophenyl)-propyl group (NPP) and used this to locally destabilize a DNA double strand, which could be transcribed after triggering with light.[7] We then used the same residue to sterically block the interaction of a protein with an aptamer and thus made its function light-triggerable.[8] In this study, we have expanded the repertoire of caged deoxynucleosides to include a caged guanosine (dG^{NPP}) and have used it to trigger the formation of highly ordered nucleic-acid secondary structures with light.[9]

Consecutive G-nucleotides in DNA or RNA molecules are known to form stable G-quadruplex structures that are created by Watson–Crick and Hoogsteen hydrogen bonding and a central monovalent cation (Figure 1a). Such nucleic acid arrangements can be found in nature and are suggested to play fundamental roles in several biological processes including modulation of telomere activity,[10] HIV infection,[11] and the activity of HIV-1 integrase[12] and human nuclear topoisomerase 1.[13] Besides telomeric regions of chromosomes, quadruplex structures have also been found to be localized in the promoter region of the c-myc gene[14] and in the immunoglobulin switch region[15] where they might be involved in the regulation of gene expression.[16] Artificially designed or in vitro selected G-quadruplex molecules have been found to interact tightly with defined proteins, such as human α-thrombin,[17] STAT3 protein[18] and nucleolin.[19] They have been successfully used as antagonists of the cognate-protein function. G-quadruplex molecules have also been investigated as potential target sites for small-molecule drugs,[20,21] for example, to inhibit telomerase activity which is up-regulated in about 85% of all cancers.[22]

In order to trigger the formation of G-quadruplexes with light we prepared the caged phosphoramidite **6** (Scheme 1). Starting with the protected deoxyguanosine (**1**) an isopropylphenoxyacetyl group was introduced to protect the exocyclic amino group (→**2**). This group can be used in the "ultramild" protecting-group strategy[23] and can be removed, for example, with dilute ammonia at RT. We chose this group because it is compatible with the previously introduced T^{NPP} residue[7] and the NPP group can also be cleaved in the usual DNA solid-phase synthesis deprotection protocol (concentrated ammonia, 65 °C).[24] After protection of the amine, the NPP group was introduced under Mitsunobu conditions (→**3**). After deprotection (→**4**) and incorporation of the DMTr-group in the 5′-position (→**5**) the phosphoramidite could be introduced (→**6**).

A common model sequence (**7**) for studying telomeres is d(AGGG(TTAGGG)$_3$), which is derived from human telomeric DNA.[21] This sequence has been shown to fold into the G-quadruplex structure as represented in Figure 1b.[25] Under various buffer conditions (high K^+ concentration) different G-quadruplex structures have recently been observed.[26] In order

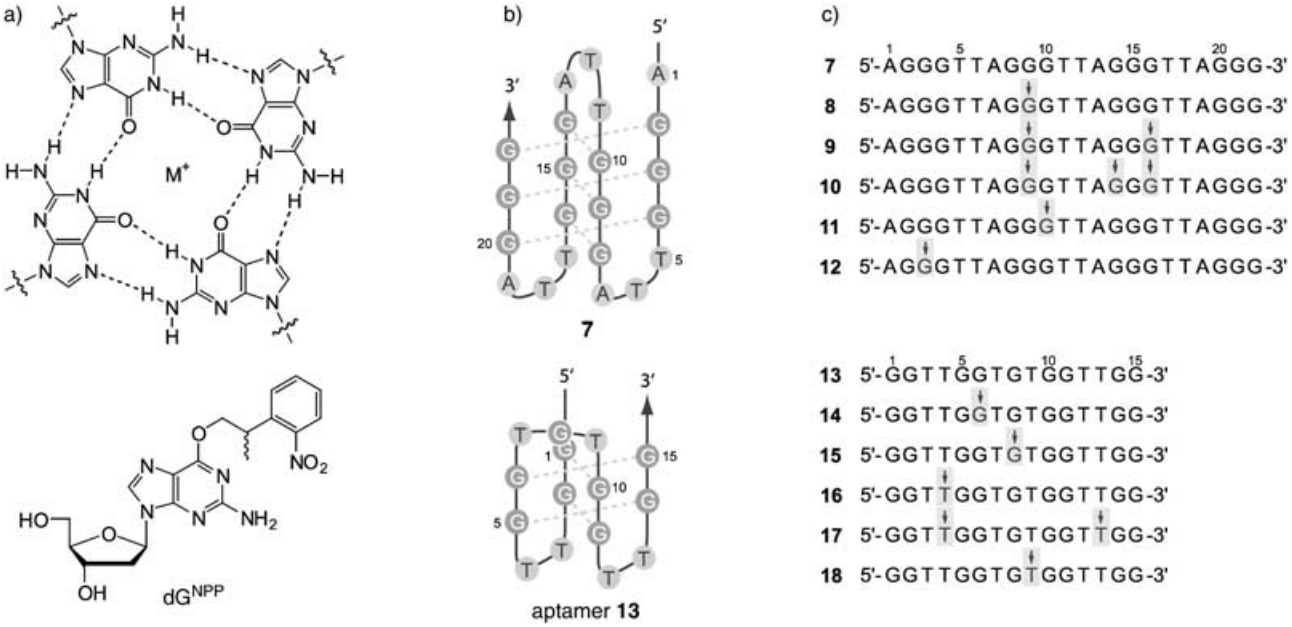
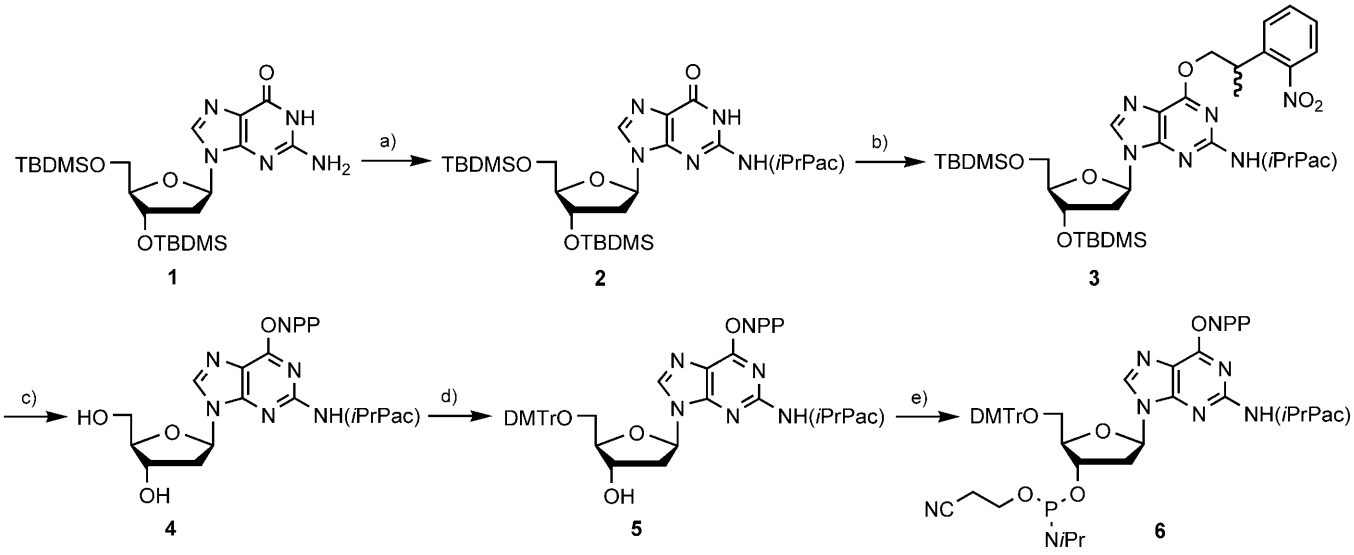


Figure 1. a) The hydrogen bonding pattern in one layer of a G-quadruplex and the caged guanosine residue dG^{NPP} used in this study. b) Schematic representation of G-quadruplex structures formed by the human telomere sequence (**7**) and aptamer **13**. c) The deoxyoligonucleotides used in this study. The positions where nucleotides have been replaced by caged counterparts are indicated with an arrow. The synthesis of T^{NPP} is described in a previous publication.[7]



Scheme 1. Synthesis of protected phosphoramidite **6** for the introduction of caged deoxyguanosine residues in deoxyoligonucleotides by using solid-phase synthesis. Reaction conditions: a) 4-*i*Pr-C_6H_4-OCH_2COCl (*i*PrPacCl), pyridine; b) 2-(2-nitrophenyl)-propan-1-ol (NPP-OH), diethylazodicarboxylate (DEAD), PPh_3; c) tetrabutylammonium fluoride, HOAc; d) dimethoxytritylchloride (DMTrCl), pyridine; e) $(NCCH_2CH_2O)(NiPr_2)PCl$, iPr_2NEt.

to study the light-induced formation of G-quadruplexes we have replaced different dG nucleotides in **7** with dG^{NPP} residues (Figure 1). CD spectroscopy was used to evaluate whether G-quadruplex structure was formed or not (Figure 2). While unmodified sequence **7** showed the expected[27] CD spectrum, replacement of one dG residue at position 9 (→**8**) was enough to prevent the formation of a G-quadruplex in solution. The CD spectrum obtained was almost identical to that of the parent oligonucleotide **7** in the absence of monovalent ions that are required for G-quadruplex formation (Figure 2a).[27] After irradiation of the caged oligonucleotide, the CD spectrum showed the signal of a normal G-quadruplex, albeit with a

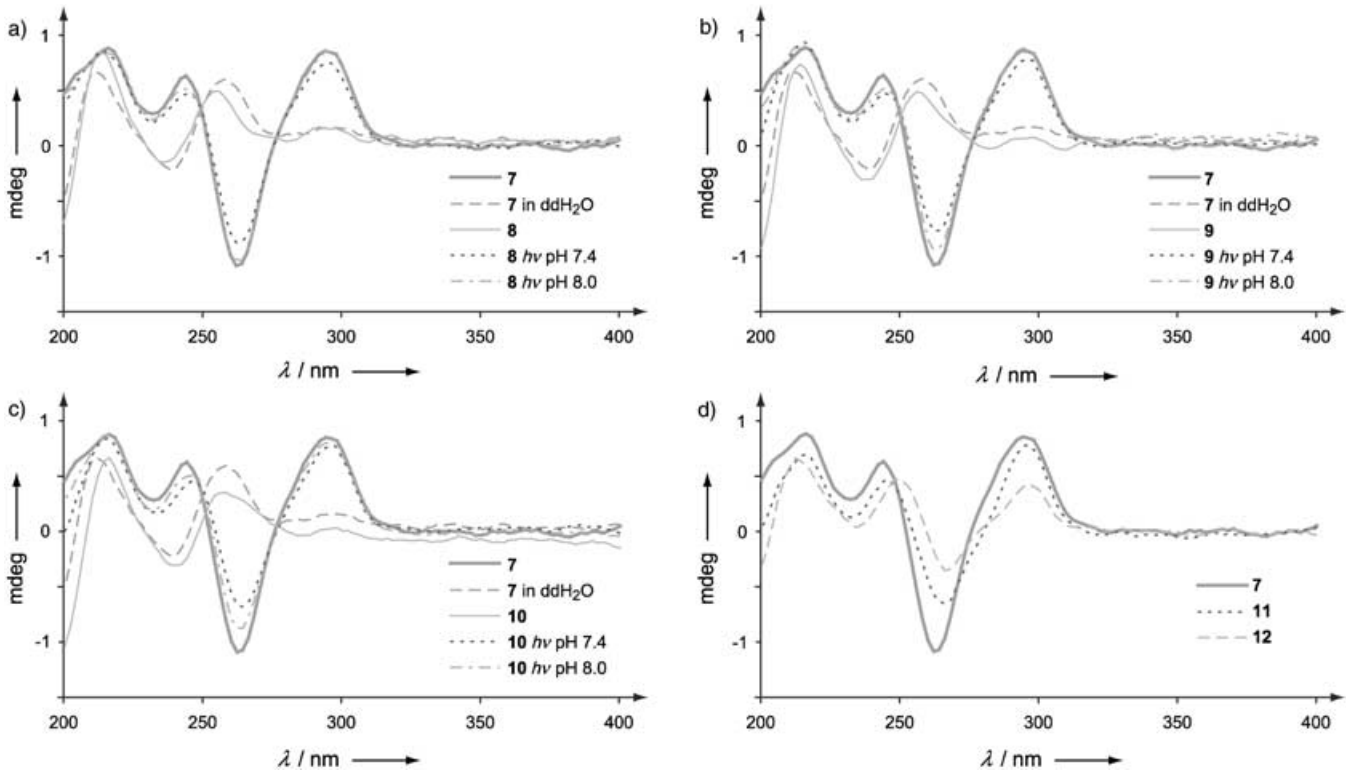


Figure 2. CD spectra of the modified telomere DNA sequences measured at RT. Unless otherwise noted the spectra were measured in PBS buffer (pH 7.4). For the curves labelled "*hν* pH 8.0", only the irradiation was performed at pH 8.0 and the resulting curves were almost identical with that of unmodified oligonucleotide **7**.

somewhat lower amplitude. A slight rise in the pH of the buffer (only during irradiation) resulted in a CD spectrum that was practically identical to that of unmodified oligonucleotide **7** (for comments see below). Introduction of more than one caged dG residue (**8**, **9**) turned out not to be necessary (Figure 2b and c), but even in these cases uncaging by irradiation occurred very efficiently.

The choice of the dG residue in the sequence is however important. Moving the modification only one position towards the 3′-end (**11**, dG^{NPP} in position 10) resulted in an oligonucleotide that still formed a G-quadruplex (Figure 2d). Qualitatively, the same result was obtained upon modification of G3 (**12**), which is close to the 5′-end (Figure 2d). Therefore, in order to destabilize a three-layer G-quadruplex, it is important to modify a residue that is both in the core of the sequence and involved in the formation of the middle layer.

The thrombin-binding DNA aptamer **13**,[17,28] which we had used in previous studies,[8] forms only a two-layer G-quadruplex structure (Figure 1). Again, modification of only one residue (in position 6) was enough to obtain a caged version (**14**) with a CD spectrum identical to that of unmodified aptamer **13** in the absence of buffer salts (Figure 3). Irradiation triggered quadruplex formation, but, in order to obtain a CD spectrum identical to that of wild-type **13**, the pH of the solution had to be significantly raised during irradiation. This demonstrates once more that the uncaging efficiency is, to some extent, sequence dependent. At present, this problem can be easily circumvented by the application of a slight excess of caged molecules; after irradiation, this results in the formation of enough uncaged material. However, future investigations will be aimed at modifying the NPP group to improve its deprotection efficiency and studying the application of different photolabile groups in these positions in order to obtain caged building blocks with clean deprotection pathways. A caged version of **13** with a modified G8 (**15**) and aptamers **16–18** with caged T^{NPP} residues, resulted in CD spectra that clearly showed an intact G-quadruplex structure.[27] In filter-binding studies with caged aptamer **14**, no interaction with the target molecule thrombin could be detected, whereas after irradiation (pH 7.4) a K_D of 80 ± 9 nM was determined, which is very similar to that of unmodified aptamer **13** (99 ± 21 nM; see Supporting Information).[8]

Thus, we have prepared a new caged dG^{NPP} nucleoside for the modification of DNA oligonucleotides and demonstrated its use for the light-triggered formation of G-quadruplex structures. This tool can, for example, be used to monitor the folding kinetics of G-quadruplex structures in real time and the spatiotemporal control of gene expression. Furthermore, it will have important implications for the development of caged aptamers because it might not always be possible to identify and sterically block the active site, as in our previous study.[8] Identifying important structural elements and modifying them so that the active conformation cannot be formed could be much easier and more efficient with the method presented here.

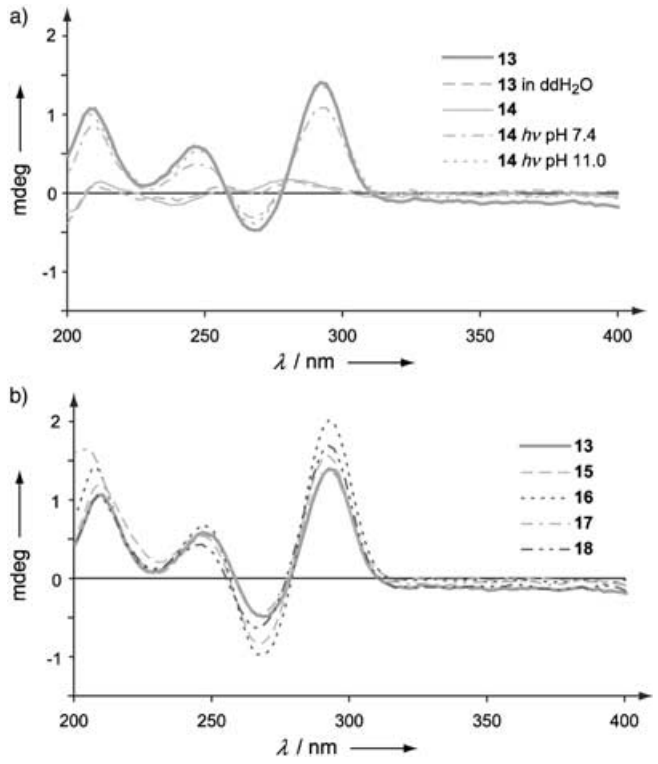


Figure 3. CD spectra of modified DNA aptamers measured at RT. Unless otherwise noted the spectra were measured in PBS buffer (pH 7.4) with $MgCl_2$ (3 mM). For the curve labelled "**14** *hν* pH 11.0" only the irradiation was performed at pH 11.0 and the resulting curve was identical with that of unmodified aptamer **13**.

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Keywords: cage compounds · DNA structures · G-quadruplexes · nucleic acids · nucleobases

[1] a) *Methods in Enzymology, Vol.* 291, *Caged Compounds* (Ed.: G. Mariott), Academic Press, London, **1998**; b) A. P. Pelliccioli, J. Wirz, *Photochem. Photobiol. Sci.* **2002**, *1*, 441 – 458.
[2] H. Ando, T. Furuta, R. Y. Tsien, H. Okamoto, *Nat. Genet.* **2001**, *28*, 317 – 325.
[3] D. Geissler, W. Kresse, B. Wiesner, J. Bendig, H. Kettnemann, V. Hagen, *ChemBioChem* **2003**, *4*, 162 – 170.
[4] W. T. Monroe, M. M. McQuain, M. S. Chang, J. S. Alexander, F. R. Haselton, *J. Biol. Chem.* **1999**, *274*, 20895 – 20900.
[5] S. Shah, S. Rangarajan, S. H. Friedman, *Angew. Chem.* **2005**, *117*, 1352 – 1356; *Angew. Chem. Int. Ed.* **2005**, *44*, 1328 – 1332.
[6] Recently, Perrin et al. presented a light-triggerable DNAzyme with a caged adenosine: R. Ting, L. Lermer, D. M. Perrin, *J. Am. Chem. Soc.* **2004**, *126*, 12720 – 12721.
[7] L. Kröck, A. Heckel, *Angew. Chem.* **2005**, *117*, 475 – 477; *Angew. Chem. Int. Ed.* **2005**, *44*, 471 – 473.
[8] A. Heckel, G. Mayer, *J. Am. Chem. Soc.* **2005**, *127*, 822 – 823.
[9] Shortly before the submission of this manuscript a paper was published in which a caged G residue was used to study the folding kinetics of

RNA: P. Wenter, B. Fürtig, A. Hainard, H. Schwalbe, S. Pitsch, *Angew. Chem.* **2005**, *117*, 2656 – 2659; *Angew. Chem. Int. Ed.* **2005**, *44*, 2600 – 2603.
[10] a) E. H. Blackburn, *Trends Biochem. Sci.* **1991**, *16*, 378 – 381; b) E. H. Blackburn, *Nature* **1991**, *350*, 569 – 573.
[11] J. R. Wyatt, T. A. Vickers, J. L. Roberson, R. W. Buckheit, Jr., T. Klimkait, E. DeBaets, P. W. Davis, B. Rayner, J. L. Imbach, D. J. Ecker, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 1356 – 1360.
[12] A. Mazumder, N. Neamati, J. O. Ojwang, S. Sunder, R. F. Rando, Y. Pommier, *Biochemistry* **1996**, *35*, 13762 – 13771.
[13] C. Marchand, P. Pourquier, G. S. Laco, N. Jing, Y. Pommier, *J. Biol. Chem.* **2002**, *277*, 8906 – 8911.
[14] a) T. Simonsson, P. Pecinka, M. Kubista, *Nucleic Acids Res.* **1998**, *26*, 1167 – 1172; b) A. Rangan, O. Y. Fedoroff, L. H. Hurley, *J. Biol. Chem.* **2001**, *276*, 4640 – 4646.
[15] P. Weisman-Shomer, M. Fry, *J. Biol. Chem.* **1993**, *268*, 3306 – 3312.
[16] H. Arthanari, P. H. Bolton, *Chem. Biol.* **2001**, *8*, 221 – 230.
[17] L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, *Nature* **1992**, *355*, 564 – 566.
[18] N. Jing, Y. Li, W. Xiong, W. Sha, L. Jing, D. J. Tweardy, *Cancer Res.* **2004**, *64*, 6603 – 6609.
[19] V. Dapic, V. Abdomerovic, R. Marrington, J. Peberdy, A. Rodger, J. O. Trent, P. J. Bates, *Nucleic Acids Res.* **2003**, *31*, 2097 – 2107.
[20] a) S. Ladame, J. A. Schouten, J. Stuart, J. Roldan, S. Neidle, S. Balasubramanian, *Org. Biomol. Chem.* **2004**, *2*, 2925 – 2931; b) J. L. Mergny, J. F. Riou, P. Mailliet, M. P. Teulade-Fichou, E. Gilson, *Nucleic Acids Res.* **2002**, *30*, 839 – 865.
[21] I. M. Dixon, F. Lopez, J. P. Esteve, A. M. Tejera, M. A. Blasco, G. Pratviel, B. Meunier, *ChemBioChem* **2005**, *6*, 123 – 132.
[22] M. Brunori, P. Luciano, E. Gilson, V. Geli, *J. Mol. Med.* **2005**, *83*, 244 – 257.
[23] "UltraMILD" phosphoramidites can be purchased from Glen Research (http://www.glenres.com). The protecting groups of their nucleobases can be removed under milder conditions compared to their regularly used counterparts.
[24] The NPP group in dG^{NPP} however is stable in concentrated ammonia at 65 °C for several hours.
[25] Y. Wang, D. J. Patel, *Structure* **1993**, *1*, 263 – 282.
[26] a) G. N. Parkinson, M. P. Lee, S. Neidle, *Nature* **2002**, *417*, 876 – 880; b) P. Hazel, J. Huppert, S. Balasubramanian, S. Neidle, *J. Am. Chem. Soc.* **2004**, *126*, 16405 – 16415.
[27] I. N. Rujan, J. C. Meleney, P. H. Bolton, *Nucleic Acids Res.* **2005**, *33*, 2022 – 2031.
[28] W. X. Li, A. V. Kaplan, G. W. Grant, J. J. Toole, L. L. Leung, *Blood* **1994**, *83*, 677 – 682.

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