JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Discovery and Synthesis of New UV-Induced Intrastrand C(4−8)G and G(8−4)C Photolesions

Martin Münzel, Claudia Szeibert, Andreas F. Glas, Daniel Globisch, and Thomas Carell*

Center for Integrated Protein Science (CIPS[M]) at the Department of Chemistry, Ludwig-Maximilians-University Munich, Butenandtstrasse 5−13, 81377 Munich, Germany

Supporting Information

ABSTRACT: UV irradiation of cellular DNA leads to the formation of a number of defined mutagenic DNA lesions. Here we report the discovery of new intrastrand C(4−8)G and G(8−4)C cross-link lesions in which the C(4) amino group of the cytosine base is covalently linked to the C(8) position of an adjacent dG base. The structure of the novel lesions was clarified by HPLC−MS/MS data for UV-irradiated DNA in combination with chemical synthesis and direct comparison of the synthetic material with irradiated DNA. We also report the ability to generate the lesions directly in DNA with the help of a photoactive precursor that was site-specifically incorporated into DNA. This should enable detailed chemical and biochemical investigations of these lesions.

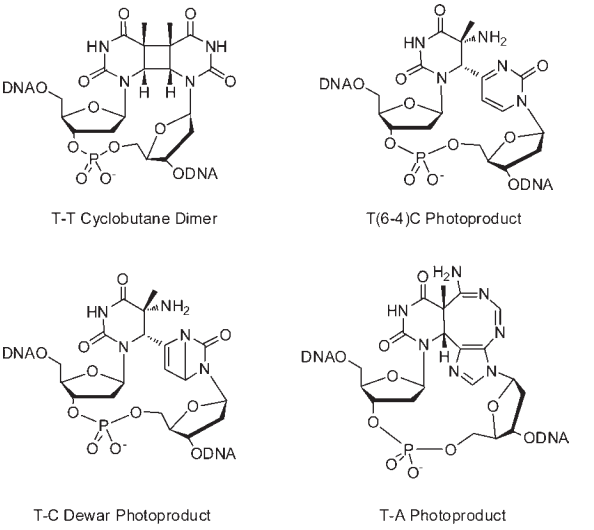


Figure 1. Chemical structures of UV-induced DNA photoproducts.

UV light is carcinogenic because it induces the formation of a series of lesions in cellular DNA that are in part highly mutagenic.[1,2] The most studied UV-induced DNA lesions are pyrimidine-derived cyclobutane pyrimidine dimers, (6−4) lesions, and their Dewar valence isomers.[3−5] Formation of these UV-induced lesions requires absorption of a UV photon by one pyrimidine nucleobase followed by reaction with a second pyrimidine base out of either the singlet or triplet state. Other than the pyrimidine−pyrimidine UV lesions, very few UV lesions have been characterized. Most notably, thymidine is able to react with an adjacent adenine to give a cycloaddition product that rearranges to produce the TA lesion (Figure 1).[6] Here we report the discovery and independent synthesis of novel UV-induced DNA lesions formed in d(CpG) or d(GpC) sequences (Figure 2). The lesions are the first of their kind and feature as a key structural element a direct link between the C(4)−NH_2 of cytosine and the C(8) carbon atom at the guanine base. As such, they are structurally related to known dG bulky adducts[7,8] and to lesions that have been identified in damaged DNA that was irradiated with X-rays[6,9] or γ-rays[10−16] or in the presence of photosensitizers.[17−19]

The lesion was discovered in experiments in which we irradiated small oligonucleotides with UV light (254 nm) in a glovebox. Whereas irradiation of oligonucleotides with a central d(TpT) or d(TpC) sequence furnished as expected the corresponding (6−4) lesions, dG-rich oligonucleotides with a central dC under exactly the same conditions[20] furnished a small but significant amount of a new oligonucleotide with an unknown lesion (for yields, see Table S1 in the Supporting Information). This lesion was found to possess an additional absorption at 350 nm. In order to characterize the lesion, we digested the DNA with a mixture of three enzymes (nuclease S1 for 3 h at 37 °C and then antarctic phosphatase and snake venom phosphodiesterase for 3 h at 37 °C) and analyzed the obtained nucleoside mixture by high-resolution HPLC−MS/MS on a Thermo Finnigan Orbitrap XL instrument (Figure 2). Indeed, aside from the signals of the canonical bases dC and dG, a new signal with a high-resolution molecular weight of 492.1717 g/mol was detected at a detection wavelength of 350 nm. Analysis of the molecular weight and MS/MS data pointed to the formation of a dC−dG cross-link with a missing central phosphodiester, which was possibly removed during the digestion procedure. On the basis of these data, we postulated for the new DNA lesion the structure of the C(4−8)G lesion depicted in Figure 2. Upon irradiation of oligonucleotides in which we had swapped the position of the dG and dC bases, the corresponding G(8−4)C product was detected. Additional experiments showed that formation of the lesions is an intrastrand process and that it occurs with the adjacent base.[17,21] We furthermore found both compounds in irradiated double strands that contained a central d(CpG) or d(GpC), which may hint at biological significance. For details, please see the Supporting Information.

In order to prove that our proposed structure is correct, we started the total synthesis of the expected digestion product without the

Received: December 15, 2010
Published: March 22, 2011

dx.doi.org/10.1021/ja111304f | J. Am. Chem. Soc. 2011, 133, 5186–5189

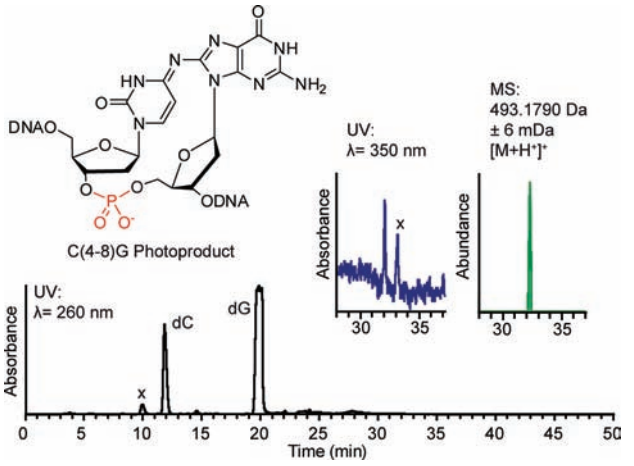


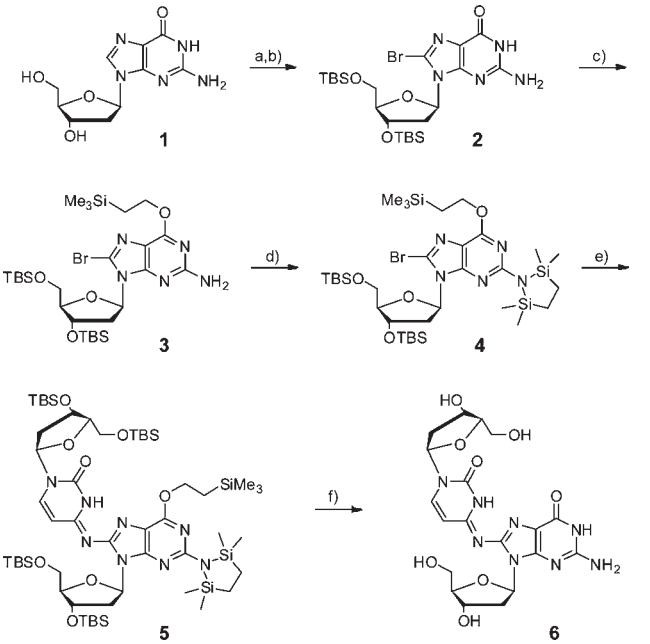
Figure 2. Structure of the C(4−8)G photolesion and the HPLC chromatogram at 260 nm after irradiation and enzymatic digestion of ODN1 [5′-d(GGC GG)-3′]. The insets show the UV chromatogram at 350 nm (blue) and the HRMS trace at 493.1790 ± 0.006 Da (green, $[M + H^+]^+$). The C−G photolesion in its dephosphorylated form can be detected in the HRMS trace at 32 min. The red PO_2^- unit was most likely cleaved out during enzymatic digestion. X = unknown products.

central phosphodiester, as shown in Scheme 1. A Buchwald−Hartwig reaction was utilized as the key step for the assembly, which was challenging because Pd-catalyzed C−N bond formations with cytosine derivatives had limited precedent.[22] For the coupling we had to protect all further reactive groups of the dG unit.[23−25] In order to streamline the synthesis, we chose to employ a protective-group strategy that allowed final removal of all of the protective groups in a single step.

The starting point of the synthesis was deoxyguanosine (**1**), which was first brominated at the C(8) position with NBS and then TBS-protected to give compound **2**.[23] Alkylation of the C(6)═O bond with TMS-ethanol under Mitsunobu conditions furnished compound **3**, which was treated with bis(chlorodimethylsilyl)ethane[22] and LiHMDS to provide the fully protected 8-bromo-dG derivative **4**. Pd-catalyzed Buchwald−Hartwig coupling of this compound with TBS-protected cytidine furnished **5** in 33% yield along with 55% recovery of **3**. Final deprotection of compound **5** yielded the desired putative lesion **6** as a dinucleotide with indeed a strong UV absorption at 350 nm (for the UV spectrum, please see Figure S2 in the Supporting Information). Co-injection of the synthetic material with digested irradiated DNA in the HPLC−MS experiment showed that the retention time, the high-resolution molecular weight, and the UV absorption characteristics of the synthetic compound and the questionable digestion product were identical (see Figure S1). We consequently conclude that the synthesized compound **6** is indeed the C(4−8)G or G(8−4)C photoproduct without the central phosphate backbone, thereby proving that the C(4−8)G lesion has the structure depicted in Figure 2.

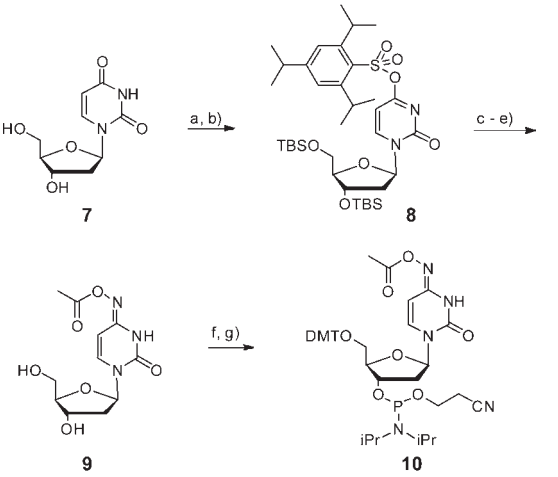
We next wanted to learn more about the mechanism leading to the formation of the novel lesions, and we also wanted to gain further support for the proposed structures. We believe that UV irradiation of cytosine-containing DNA leads to the formation of the cytosine base in the excited triplet state. In this state, the dC(4)−NH_2 group is known to possess substantial spin density,[26−28] which for example allows the formation of the well-studied deoxycytosine-containing (6−4) lesions.[4] Indeed, the observation that the novel lesion forms after irradiation of DNA preferentially under exclusion of oxygen in a

Scheme 1. Total Synthesis of the C−G Photoproducts without Central Phosphate[a]



[a] Conditions: (a) NBS, 94%; (b) TBS-Cl, imid., 98%; (c) TMS-EtOH, PPh_3, DIAD, 75%; (d) $(ClMe_2SiCH_2)_2$, LiHMDS, 79%; (e) TBS-dC, (±)-BINAP, LiHMDS, $Pd_2(dba)_3$, 33%; (f) TBAF, 50%.

Scheme 2. Synthesis of $^{4\text{-NHOH}}$dC Phosphoramidite 10[a]



ODN2: 5' AGG TC*G GC 3'

[a] Conditions: (a) TBS-Cl, imid., 83%; (b) triisopropylbenzenesulfonyl chloride, NaH, 99%; (c) NH_3OHCl, DBU, 34%; (d) Ac_2O, 97%; (e) HF·pyr, pyridine, 63%; (f) DMT-Cl, pyridine, 83%; (g) bis(diisopropylamino)(2-cyanoethoxy)phosphine, diisopropyltetrazolide, 55%. C* = $^{4\text{-NHOH}}$dC.

glovebox is a strong hint that a triplet state is involved in lesion formation. The dC(4)−NH_2 "radical" could in the next step attack the C(8) position of the adjacent dG base, in accord with the well-known fact that radicals tend to react with the dG base at C(8) to give, for example, the mutagenic lesion 8-oxo-dG.[29−31] Final oxidation of

dx.doi.org/10.1021/ja111304f |J. Am. Chem. Soc. 2011, 133, 5186–5189

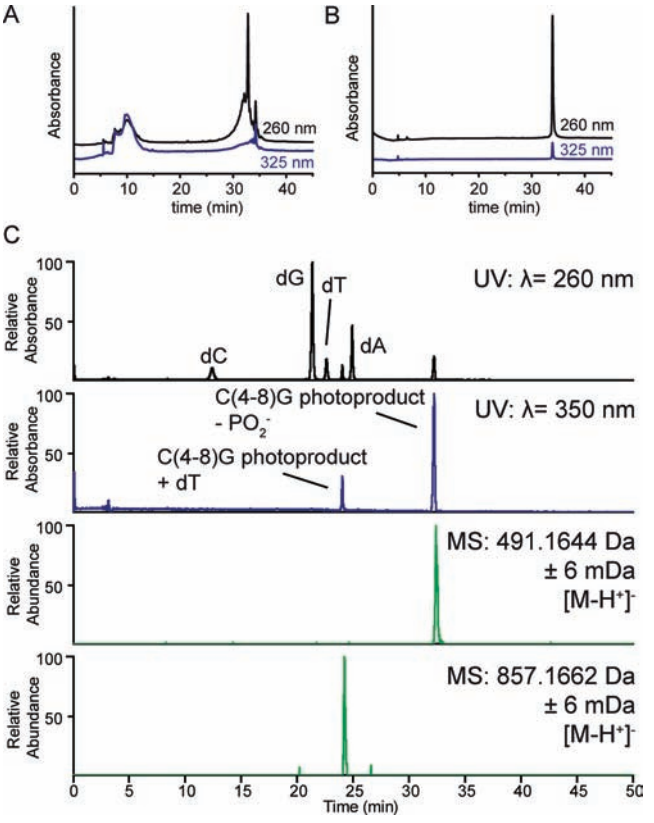


Figure 3. (A) HPLC chromatogram of ODN2 directly after irradiation. (B) HPLC chromatogram of purified ODN2 containing the C(4−8)G photolesion. (C) HPLC chromatogram and MS traces (negative-ion mode) after irradiation, HPLC purification, and enzymatic digestion of ODN2.

the reaction product by traces of oxygen would then furnish the highly conjugated (4−8) lesion. On the basis of the reaction conditions, we do not believe that the formation of the lesion is initiated by a radical such as HO· or CO_3·, as was proposed for similar G(8−3)T, G(8−5)C, and G(8−5)mC lesions.[17,32,33] For a mechanistic proposal, please see the Supporting Information. In order to gain support for this hypothesis, we prepared a dC(4)−NH· radical dC precursor and incorporated it into DNA. We chose the hydroxylamine-dC derivative,[34,35] which should give the dC(4)−NH· radical upon irradiation by cleavage of the rather weak N−O bond.

The synthesis of the dC hydroxylamine precursor phosphoramidite was accomplished in seven steps, as shown in Scheme 2. After TBS protection of deoxyuridine **7**, the 4-position was activated as a sulfonate to yield compound **8**. Nucleophilic aromatic substitution with hydroxylamine hydrochloride and subsequent acetylation and deprotection with HF in pyridine furnished compound **9**. Free nucleoside **9** was converted to the phosphoramidite **10** by DMT protection and subsequent phosphitylation. The incorporation into oligonucleotides was possible using standard phosphoramidite chemistry. For the lesion formation experiment, we prepared the oligonucleotide ODN2 in which the precursor was situated next to a 3′-dG base and a 5′-dT (Scheme 2). Using this setup, we intended to get direct insight into the reactivity of dC(4)−NH· (dG vs dT). Irradiation of ODN2 with a mercury lamp for 14 h gave only one new distinct oligonucleotide with a characteristic absorption at >300 nm (Figure 3a). The irradiated DNA strand was purified (Figure 3b), digested, and analyzed by HPLC−MS (Figure 3c). In the digested sample, we observed next to the canonical bases two new signals, both of which had the unusual long-wavelength absorption. Both compounds had molecular weights consistent with selective formation of the expected C(4−8)G lesion. One signal corresponded to the already known C(4−8)G compound with the open backbone. The second signal originated from an incomplete digestion product consisting of the C(4−8)G lesion including the phosphate plus an adjacent thymidine. Surprisingly, no formation of a d(TC) adduct was observed, showing the preferred reaction of dC(4)−NH· with an adjacent guanine. This result supports the idea that a UV-induced dC(4)−N-centered cytosine radical can attack the C(8) position of an adjacent dG base to form the CG photoproducts. The hydroxylamine-dC precursor furthermore allows the direct production of oligonucleotides containing the novel C(4−8)G lesion. It can be site-specifically incorporated in sufficient amounts and excellent purity. This will enable detailed biochemical investigation of the unusual new intrastrand cross-link lesions.[36] Furthermore, our total synthesis allows the preparation of isotope-labeled lesion analogues for direct quantification of the compound in cellular DNA.[37]

In summary, we have reported the discovery of new UV-induced lesions that are formed by oxidation of an initially formed dC−dG cross-link to give a highly conjugated heterocyclic ring system. The new lesions are formed in d(CpG) and d(GpC) sequences in both single strands and duplexes. They feature new absorption bands above 350 nm, which allow ready detection. The lesions can easily be site-specifically prepared in DNA by irradiation of DNA containing a hydroxylamine-dC precursor situated next to a dG base.

■ ASSOCIATED CONTENT

Supporting Information. Irradiation of oligonucleotides, preparation of compounds **1**−**10**, oligonucleotide synthesis and purification, enzymatic digestion, and detailed HPLC−MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
thomas.carell@cup.uni-muenchen.de

■ ACKNOWLEDGMENT

We thank Amra Mulahasanovic (LMU Munich) for preparative work. Financial support was obtained from the DFG (CA 275/8-4 and SFB749). M.M. thanks the Fonds der Chemischen Industrie for a Kekulé Fellowship.

■ REFERENCES

(1) Cadet, J.; Sage, E.; Douki, T. *Mutat. Res.* **2005**, *571*, 3–17.
(2) Friedel, M. G.; Cichon, M. K.; Carell, T. *CRC Handbook of Organic Photochemistry and Photobiology*, 2nd ed.; CRC Press: Boca Raton, FL, 2004.
(3) Glas, A. F.; Kaya, E.; Schneider, S.; Heil, K.; Fazio, D.; Maul, M. J.; Carell, T. *J. Am. Chem. Soc.* **2010**, *132*, 3254–3255.
(4) Heil, K.; Pearson, D.; Carell, T. *Chem. Soc. Rev.* [Online early access]. DOI: 10.1039/C000407N. Published Online: Nov 15, 2010.
(5) Cadet, J.; Vigni, P. In *Photochemistry and the Nucleic Acids*; Morrison, H., Ed.; Bioorganic Photochemistry, Vol. 1; Wiley: New York, 1990; Chapter 1.
(6) Zhao, X.; Taylor, J. S. *Nucleic Acids Res.* **1996**, *24*, 1561–1565.

(7) Schorr, S.; Schneider, S.; Lammens, K.; Hopfner, K. P.; Carell, T. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 20720–20725.
(8) Hoffmann, G. R.; Fuchs, R. P. *Chem. Res. Toxicol.* **1997**, *10*, 347–359.
(9) Box, H. C.; Dawidzik, J. B.; Budzinski, E. E. *Free Radical Biol. Med.* **2001**, *31*, 856–868.
(10) Delatour, T.; Douki, T.; Gasparutto, D.; Brochier, M. C.; Cadet, J. *Chem. Res. Toxicol.* **1998**, *11*, 1005–1013.
(11) Gu, C.; Wang, Y. *Biochemistry* **2004**, *43*, 6745–6750.
(12) Hong, H.; Cao, H.; Wang, Y. *Nucleic Acids Res.* **2007**, *35*, 7118–7127.
(13) Romieu, A.; Bellon, S.; Gasparutto, D.; Cadet, J. *Org. Lett.* **2000**, *2*, 1085–1088.
(14) Su, D. G.; Taylor, J. S.; Gross, M. L. *Chem. Res. Toxicol.* **2010**, *23*, 474–479.
(15) Zeng, Y.; Wang, Y. *J. Am. Chem. Soc.* **2004**, *126*, 6552–6553.
(16) Zhang, Q.; Wang, Y. *J. Am. Chem. Soc.* **2003**, *125*, 12795–12802.
(17) Crean, C.; Uvaydov, Y.; Geacintov, N. E.; Shafirovich, V. *Nucleic Acids Res.* **2008**, *36*, 742–755.
(18) Liu, Z.; Gao, Y.; Wang, Y. *Nucleic Acids Res.* **2003**, *31*, 5413–5424.
(19) Liu, Z.; Gao, Y.; Zeng, Y.; Fang, F.; Chi, D.; Wang, Y. *Photochem. Photobiol.* **2004**, *80*, 209–215.
(20) Maul, M. J.; Barends, T. R.; Glas, A. F.; Cryle, M. J.; Domratcheva, T.; Schneider, S.; Schlichting, I.; Carell, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 10076–10080.
(21) Douki, T.; Laporte, G.; Cadet, J. *Nucleic Acids Res.* **2003**, *31*, 3134–3142.
(22) Takamura-Enya, T.; Enomoto, S.; Wakabayashi, K. *J. Org. Chem.* **2006**, *71*, 5599–5606.
(23) Gillet, L. C.; Schärer, O. D. *Org. Lett.* **2002**, *4*, 4205–4208.
(24) Lakshman, M. K. *J. Organomet. Chem.* **2002**, *653*, 234–251.
(25) Wang, Z.; Rizzo, C. J. *Org. Lett.* **2001**, *3*, 565–568.
(26) Hawkins, C. L.; Davies, M. J. *Chem. Res. Toxicol.* **2001**, *14*, 1071–1081.
(27) Hawkins, C. L.; Davies, M. J. *Chem. Res. Toxicol.* **2002**, *15*, 83–92.
(28) Naumov, S.; Barthel, A.; Reinhold, J.; Dietz, F.; Geimer, J.; Beckert, D. *Phys. Chem. Chem. Phys.* **2000**, *2*, 4207–4211.
(29) Cadet, J.; Douki, T.; Ravanat, J. L. *Nat. Chem. Biol.* **2006**, *2*, 348–349.
(30) Hsu, G. W.; Ober, M.; Carell, T.; Beese, L. S. *Nature* **2004**, *431*, 217–221.
(31) Schärer, O. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 2946–2974.
(32) Box, H. C.; Budzinski, E. E.; Dawidzik, J. B.; Wallace, J. C.; Iijima, H. *Radiat. Res.* **1998**, *149*, 433–439.
(33) Cao, H.; Wang, Y. *Nucleic Acids Res.* **2007**, *35*, 4833–4844.
(34) Münzel, M.; Lercher, L.; Müller, M.; Carell, T. *Nucleic Acids Res.* **2010**, *38*, e192.
(35) Freese, E.; Bautz, E.; Freese, E. B. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 845–855.
(36) Gu, C.; Wang, Y. *Biochemistry* **2005**, *44*, 8883–8889.
(37) Münzel, M.; Globisch, D.; Brückl, T.; Wagner, M.; Welzmiller, V.; Michalakis, S.; Müller, M.; Biel, M.; Carell, T. *Angew. Chem., Int. Ed.* **2010**, *49*, 5375–5377.