ORGANIC LETTERS

2008
Vol. 10, No. 2
249−251

Synthesis of Modified DNA by PCR with Alkyne-Bearing Purines Followed by a Click Reaction

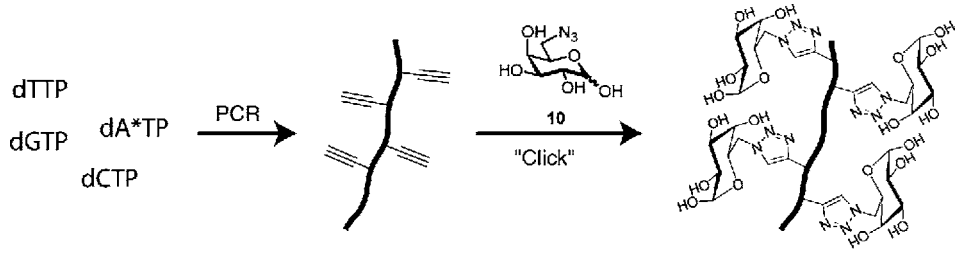
Philipp M. E. Gramlich, Christian T. Wirges, Johannes Gierlich, and Thomas Carell*

Department of Chemistry, Ludwig-Maximilians University Munich, Butenandtstr. 13, D-81377 Munich, Germany

thomas.carell@cup.uni-muenchen.de

Received October 26, 2007

ABSTRACT



Alkyne-bearing deazapurine triphosphates were prepared and successfully incorporated into DNA using the polymerase chain reaction (PCR). The obtained alkyne-labeled DNA was successfully used in a click reaction with galactose azide.

The utilization of DNA for the assembly of functional materials has received considerable interest in recent years.[1] To this end, modified DNA was created featuring a multitude of novel functions which, for example, allows one to accelerate the detection of an analyte,[2] to sequence DNA,[3] to create DNA- and RNA-based receptors (aptamers),[4] or to assemble them into novel nanomaterials.[5] In order to introduce the modifications in a highly controllable manner in long DNA strands, the exchange of the natural triphosphates by chemically modified building blocks in enzymatic reactions such as the PCR reaction is the most common approach. This method is, however, not generally applicable because it cannot be predicted if and how a specific triphosphate is accepted by different DNA polymerases.[6,7] We therefore decided to develop a two-step procedure in which a nucleoside precursor is incorporated into DNA by PCR, which can be subsequently converted into a variety of derivatives. For a similar approach using the Staudinger ligation, see ref 8. For the functionalization step, we exploited the recently described Cu-catalyzed Huisgen−Meldal−Sharpless reaction,[9] termed click reaction. We already used this chemistry to efficiently modify DNA-bearing alkyne functionalities at thymidines or cytidines.[2,10,11] We herein

* To whom correspondence should be addressed. Fax: +49 (0)89 2180 77756. Tel: +49 (0)89 2180 77750.

(1) (a) Condon, A. *Nat. Rev. Genet.* **2006**, *7* (7), 565−575. (b) Feldkamp, U.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2006**, *45* (12), 1856−1876.

(2) Hammond, D. M.; Manetto, A.; Gierlich, J.; Azov, V. A.; Gramlich, P. M. E.; Burley, G. A.; Maul, M.; Carell, T. *Angew. Chem., Int. Ed.* **2007**, *46* (22), 4184−4187.

(3) Seo, T. S.; Bai, X.; Kim, D. H.; Meng, Q.; Shi, S.; Ruparel, H.; Li, Z.; Turro, N. J.; Ju, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (17), 5926−5931.

(4) Sidorov, A. V.; Grasby, J. A.; Williams, D. M. *Nucleic Acids Res.* **2004**, *32* (4), 1591−1601.

(5) (a) Braun, E.; Eichen, Y.; Silvan, U.; Ben-Yoseph, G. *Nature* **1998**, *391*, 775−778. (b) Keren, K.; Krueger, M.; Gilad, R.; Ben-Yoseph, G.; Sivan, U.; Braun, E. *Science* **2002**, *297* (5578), 72−75. (c) Gu, Q.; Cheng, C.; Gonela, R.; Suryanarayanan, S.; Anabathula, S.; Dai, K.; Haynie, D. T. *Nanotechnology* **2006**, *17*, R14−R25.

(6) Jäger, S.; Rasched, G.; Kornreich-Leshem, H.; Engeser, M.; Thum, O.; Famulok, M. *J. Am. Chem. Soc.* **2005**, *127* (43), 15071−15082.

(7) (a) Kuwahara, M.; Nagashima, J.-i.; Hasegawa, M.; Tamura, T.; Kitagata, R.; Kazuo, H.; Hososhima, S.-i.; Kasamatsu, T.; Ozaki, H.; Sawai, H. *Nucleic Acids Res.* **2006**, *34* (19), 5383−5394. (b) Gourlain, T.; Sidorov, A.; Mignet, N.; Thorpe, S. J.; Lee, S. E.; Grasby, J. A.; Williams, D. M. *Nucleic Acids Res.* **2001**, *29* (9), 1898−1905.

(8) (a) Wang, C. C. Y.; Seo, T. S.; Li, Z.; Ruparel, H.; Ju, J. *Bioconjugate Chem.* **2003**, *14* (3), 607−701. (b) Weisbrod, S. H.; Marx, A. *Chem. Commun.* **2007**, *18*, 1828−1830.

10.1021/ol7026015 CCC: $40.75
Published on Web 12/13/2007

wish to report the extension of the technology to the purine bases dA and dG.

In order to limit structural perturbation of the DNA duplex to a minimum, we placed the alkyne group in the major groove.[6,12] To achieve this, we prepared the alkyne-bearing derivatives of the 7-deazapurine bases 7-deaza-dA and 7-deaza-dG (Figure 1). The adenine building block was

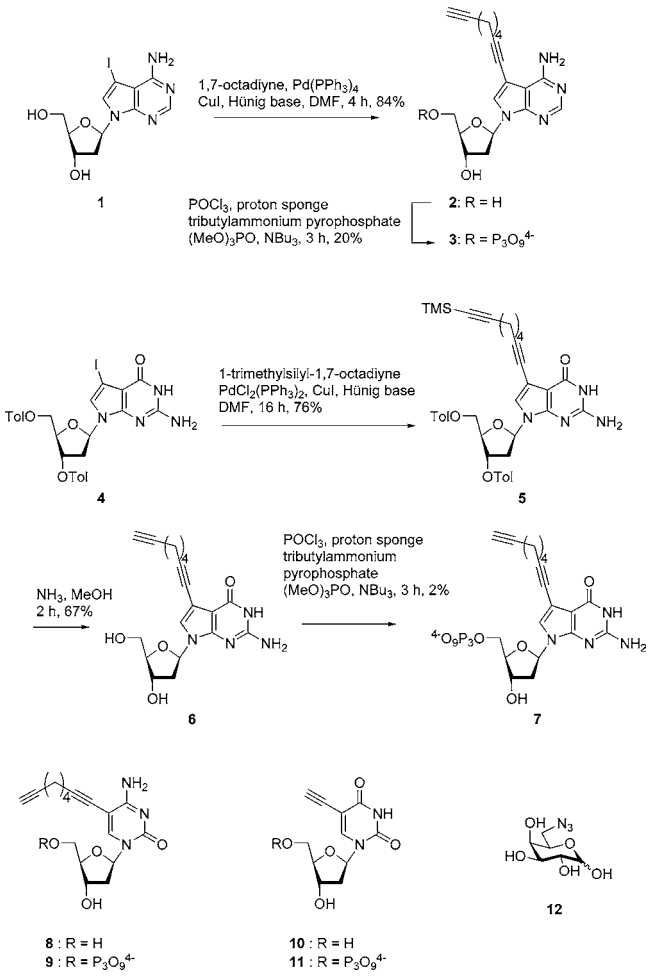


Figure 1. Syntheses of the triphosphates **3** and **7**. The alkyne-bearing pyrimidine triphosphates **9** and **11** as well as the galactose azide **12** were reported previously.

synthesized by a Sonogashira coupling on the unprotected 7-deaza-7-iodo-2′-deoxyadenosine **1** to give the free nucleoside **2**.[13,14] The synthesis of the triphosphate **3** was achieved by the method of Kovacs et al.[15] The corresponding 7-deaza-7-iodo-2′-deoxyguanosine **4** was synthesized in 10 steps according to literature procedures.[14] Sonogashira coupling of **4** with 1-TMS-protected 1,7-octadiyne gave the key intermediate **5** in good yield. After deprotection of the alkyne and cleavage of the sugar protecting groups to **6**, the monomer was successfully converted into triphosphate **7**,[15] suitable for enzymatic incorporation.

To examine how the triphosphates **3** and **7** are accepted by polymerases, we first performed primer extension studies. To this end, a fluorescently labeled primer was hybridized to a 30mer template. The elongation of the primer was followed using PAGE. In the initial test runs, we used two different templates containing each of the canonical bases at least one time.[16] As expected from literature precedents,[6] the unnatural alkyne-bearing purines **3** and **7** were able to fully replace the canonical bases dA and dG in typical primer extension experiments. In further experiments, we could also replace the nucleobases dT and dC by the alkyne-modified bases **9** and **11**, allowing us to prepare a DNA derivative in which each nucleobase carries an alkyne-bearing functional group. In all cases, we obtained a clean, full-length product, proving that all triphosphates were efficiently incorporated (Supporting Information).

Encouraged by this result, we started to incorporate our building blocks **3** and **7** using PCR. To this end, we used a 300mer template DNA to create two modified DNA strands, DNA300·**2** (dATP replaced by **3**) and DNA300·**6** (dGTP replaced by **7**). Detection of the PCR products obtained with the building block **7** turned out to be difficult because the ethidium bromide fluorescence is typically quenched by deazapurine derivatives.[17] We found that the PCR product DNA300·**2**, however, was stainable with ethidium bromide and also with SYBR green. Detection of DNA300·**6** required the use of a fluorescein-labeled primer strand. With the help of the KOD XL polymerase, we could indeed prepare DNA300·**2** and DNA300·**6** efficiently without the use of any additives (Figure 2). The obtained amplicons comprise 170 (DNA300·**2**) or 103 (DNA300·**6**) alkynes attached to the purine bases. Simple screenings of the different temperature steps (annealing, elongation, and denaturation) of the PCR cycle were sufficient to achieve this goal. An attempted PCR incorporation of all four alkyne-modified triphosphates into a 300mer DNA unfortunately failed. In order to investigate the structure of the modified DNA in more detail, we performed UV and CD measurements (Supporting Information). Both DNA strands exhibited an additional absorption band at longer wavelengths due to conjugation of the nucleobase to the internal alkyne. This observation is in accord with previous results obtained by us.[11] This additional

(9) (a) Huisgen, R. *1,3-Dipolar Cycloaddition Chemistry*; Wiley: New York, 1984; pp 1−176. (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67* (9), 3057−3064. (c) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021.

(10) (a) Gierlich, J.; Burley, G. A.; Gramlich, P. M. E.; Hammond, D. M.; Carell, T. *Org. Lett.* **2006**, *8* (17), 3639−3642. (b) Burley, G. A.; Gierlich, J.; Mofid, M. R.; Nir, H.; Tal, S.; Eichen, Y.; Carell, T. *J. Am. Chem. Soc.* **2006**, *128* (5), 1398−1399.

(11) Gierlich, J.; Gutsmiedl, K.; Gramlich, P. M. E.; Schmidt, A.; Burley, G.; Carell, T. *Chem.—Eur. J.* **2007**, *13*, 9486−9494.

(12) Capek, P.; Cahová, H.; Pohl, R.; Hocek, M.; Gloeckner, C.; Marx, A. *Chem.—Eur. J.* **2007**, *13*, 6196−6203.

(13) (a) Seela, F.; Zulauf, M. *Synthesis* **1996**, 726−730. (b) Seela, F.; Sirivolu, V. R., *Chem. Biodiversity* **2006**, *3*, 509−514.

(14) (a) Davoll, J. *J. Chem. Soc.* **1960**, *1960*, 130−138. (b) Buhr, C. A.; Wagner, R. W.; Grant, D.; Froehler, B. C. *Nucleic Acids Res.* **1996**, *24* (15), 2974−2980.

(15) Kovacs, T.; Ötvös, L. *Tetrahedron Lett.* **1988**, *29* (36), 4525−4528.

(16) Held, H. A.; Benner, S. A. *Nucleic Acids Res.* **2002**, *30* (17), 3857−3869.

(17) (a) Li, H.; Seela, F. *Nucleosides Nucleotides* **2005**, *24* (5−7), 865−868. (b) Weiss, J.; Zucht, H.-D.; Forssmann, W.-G. *PCR Methods Appl.* **1994**, *4*, 124−125. (c) Latimer, L. J. P.; Lee, J. S. *J. Biol. Chem* **1991**, *266* (21), 13849−13851.

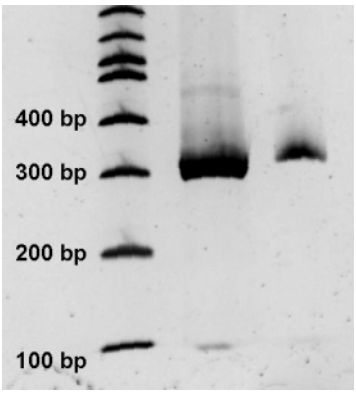


Figure 2. Five percent PAGE of the PCR amplicons 300DNA·**2** (lane 2) and 300DNA·**6** (lane 3). Lane 1 contains a 100 bp DNA ladder.

band is also visible in the CD spectra, which exhibit an extra minimum at 310 nm. The overall shape of the curve is similar to a typical B-form DNA, which is an indication that both DNA strands DNA300·**2** and DNA300·**6** adopt a B-type conformation as duplexes.

In order to prove the presence of the modifications in the DNA strands, we performed enzymatic digests of 300DNA·N (unmodified), 300DNA·**2**, and 300DNA·**6** (Figure 3). The

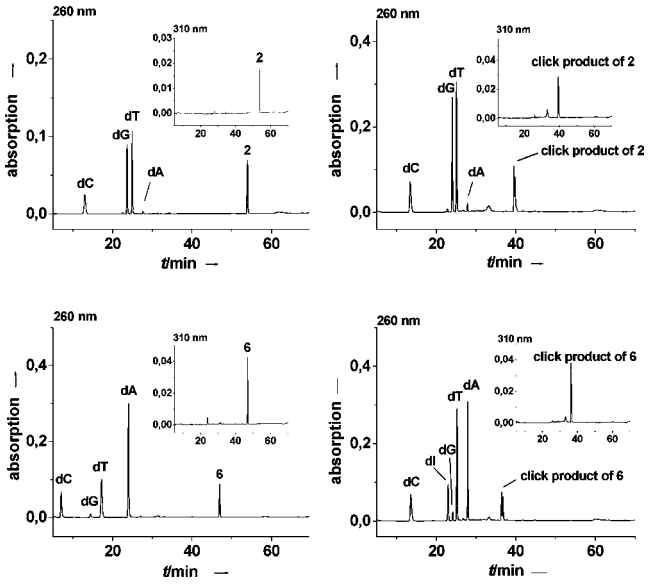


Figure 3. HPLC chromatograms of the enzymatic digest of DNA300·**2** (top) and DNA300·**6** (bottom) before (left) and after (right) the click reaction with galactose azide **12**. The inset shows the HPLC trace at 310 nm. Deoxyinosine (dI) is formed by deamination of dA during the enzymatic digest. The click products form double peaks due to anomerization of the sugar moiety.

DNA was treated with nuclease P1, calf spleen phosphodiesterase, snake venom phosphodiesterase, and alkaline phosphatase. The obtained mixture of nucleosides was separated by HPLC, and the modified bases **2** and **6** were co-injected. These spectra show that the DNA indeed contains the modified purine bases **2** and **6**. Only a small amount of the unsubstituted purine nucleosides dA and dG were detected, which derive from the commercial primers that were used for the PCR reactions. This result proves that our triphosphates were efficiently incorporated into 300DNA·**2** and 300DNA·**6**. Further proof for the presence of the modified bases was obtained from ESI-MS/MS and UV spectra of the eluting compounds.

Having achieved the successful incorporation of **3** and **7** into the PCR amplicons, we wanted to know if the modified DNA features sequence changes. To this end, we used 300DNA·**2** and 300DNA·**6** as templates for a PCR reaction with the natural dNTPs and either KOD XL or Deep Vent exo$^-$ polymerase. The resulting amplicons were sequenced (Supporting Information). We observed that the 300DNA·N strands retained the original sequence, proving that the modified bases do not reduce the polymerase fidelity in our PCR reaction but are faithfully copied.

In order to prove the proficiency of the alkyne-containing DNA in the click reaction, we used DNA·**2** and DNA·**6** as a reaction partner in the Cu(I)-catalyzed click reaction with galactose azide **12**. CuBr and TBTA ligand[18] were added to perform the reaction. The reaction products were purified from excess reactants by simple ethanol precipitation and were digested enzymatically. HPLC chromatography of the resulting nucleoside mixtures combined with UV and MS/MS analysis clearly showed the quantitative yield of the postsynthetic click functionalization, which proceeds without formation of any detectable side product. No traces of unreacted alkynes could be found (Figure 3).

In summary, we present novel alkyne-bearing purines, which can be incorporated into DNA via a PCR reaction. These building blocks act as selective reaction partners in the click reaction with azides. Quantitative conversion of more than 100 alkynes in DNA was again observed.

Acknowledgment. We thank BASF AG and the Deutsche Forschungsgemeinschaft (SFB 486) for financial support. P.M.E.G. thanks the Cusanuswerk for a Ph.D. scholarship. C.T.W. and J.G. thank the Fonds of the Chemical Industry for Ph.D. scholarships. We are indebted to Markus Spallek, Ana Varja, Veronika Ehmke, Markus Horn. and Simon Künzel from the LMU Munich for their synthetic contributions.

Supporting Information Available: Detailed experimental protocols and spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL7026015

(18) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6* (17), 2853−2855.