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High-Density DNA Functionalization by a Combination of Cu-Catalyzed and Cu-Free Click Chemistry

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Abstract: We report the regioselective Cu-free click modification of styrene functionalized DNA with nitrile oxides. A series of modified oligodeoxynucleotides (nine base pairs) was prepared with increasing styrene density. 1,3-Dipolar cycloaddition with nitrile oxides allows the high density functionalization of the styrene modified DNA directly on the DNA solid support and in solution. This click reaction proceeds smoothly even directly in the DNA synthesizer and gives exclusively 3,5-disubstituted isoxazolines. Additionally, PCR products (300 and 900 base pairs) were synthesized with a styrene triphosphate and KOD XL polymerase. The click reaction on the highly modified PCR fragments allows functionalization of hundreds of styrene units on these large DNA fragments simultaneously. Even sequential Cu-free and Cu-catalyzed click reaction of PCR amplicons containing styrene and alkyne carrying nucleobases was achieved. This new approach towards high-density functionalization of DNA is simple, modular, and efficient.

Keywords: click chemistry · DNA labeling · nitrile oxides · nucleotides · polymerase chain reaction

Introduction

The precise functionalization of oligonucleotides is of paramount importance in the research areas of DNA based nano- and biotechnology.[1] In both fields the extraordinary self-assembly and self-recognition capability of oligonucleotides are either utilized to organize nanomaterials or to bind to specific medicinally relevant oligonucleotide targets.[2] In nanotechnological applications, DNA is typically used to assemble nanoparticles, inorganic clusters or enzymes in one, two, and now even three dimensions.[3] DNA in biomolecular applications is mostly utilized as sequence specific probe molecule. Functionalized with, for example, fluorescence donors and acceptors or redox probes, the functionalized oligonucleotides hybridize specifically with relevant DNA or RNA molecules to enable their detection and/or isolation for diagnostic purposes.[4] For all these applications, reporter and anchor molecules or molecules, which carry a certain function such as electrical conductivity or magnetism have to be linked to the DNA or RNA molecule.[5] This is typically achieved in two different ways. The molecules can be directly inserted into DNA, for example, as phosphoramidite building blocks for solid phase oligonucleotide assembly or as triphosphates via PCR. Alternatively, and particularly important for sensitive molecules, an anchor molecule can be inserted into the oligonucleotide during DNA synthesis that enables the postsynthetic attachment of the desired molecule to the DNA strand.[6] The most prominent method currently available for the functionalization of DNA is the Cu^{I}-catalyzed alkynes and azides cycloaddition[7] developed by Meldal[8] and Sharpless.[9] Others and us reported the use of the Cu^{I}-catalyzed click reaction for the high density functionalization of oligonucleotides.[10] The procedure requires incorporation of alkyne-carrying nucleobase either as phosphoramidites or triphosphates into oligonucleotides and the postsynthetic functionalization of the corresponding alkyne-DNA with various azides.[11]

Although the Cu^{I}-catalyzed reaction of alkynes with azides was found to be high yielding and efficient, the presence of Cu^{I} can cause problems, particularly when the synthesis of DNA–protein conjugates is desired.[12] Up to now several Cu-free click reactions were developed such as the reaction of azides with strained cycloalkynes pioneered by Bertozzi,[13] the reaction of tetrazines with strained alkenes,[14] reaction of nitrile oxides with alkynes,[15] reaction of oxanorbornadienes with azides,[16] and a photoclick reaction discovered by Lin.[17] We recently reported the use of norbornene-functionalized oligonucleotides, in which the

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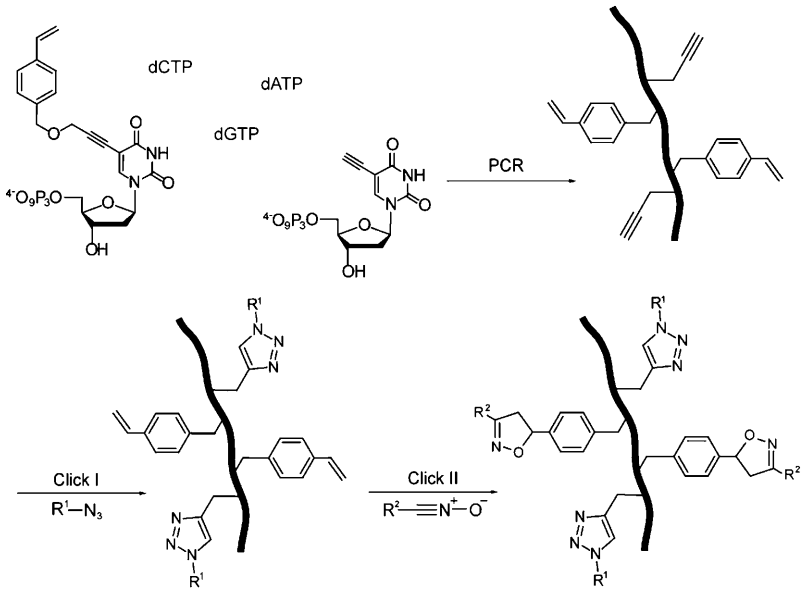
strained norbornene double bond was found to react quickly with in situ prepared nitrile oxides.[18] However, this reaction gives for each norbornene reaction site two regioisomers, which makes the oligonucleotide purification complicated.

Here we report that styrene functionalized oligonucleotides react fast and smooth with nitrile oxides. This reaction furnishes exclusively one regioisomer, the 3,5-disubstituted isoxazolines. A styrene modified triphosphate was found to be readily accepted by polymerase so that it can be inserted into long gene fragments by PCR. Finally we found that it is possible to insert alkyne- and styrene-functionalized building blocks into PCR amplicons simultaneously.[19] The reactions of azides with the alkyne sites in DNA (Cu-catalyzed) and of the nitrile oxides with the styrene units (Cu-free) were found to be orthogonal so that alkyne–styrene modified PCR fragments can be high density functionalized with two different sets of molecules as depicted in Scheme 1.

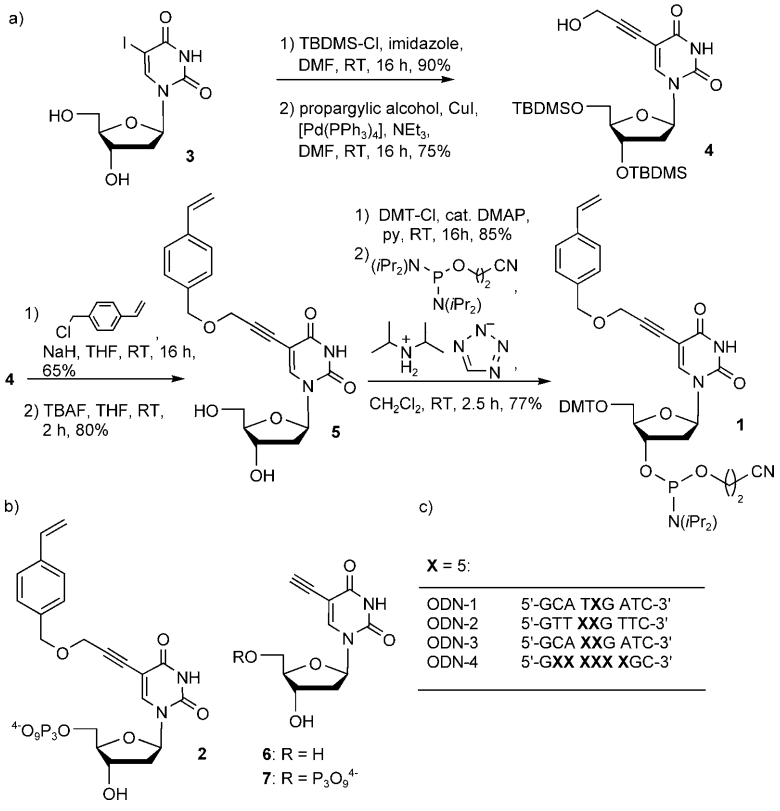
Results and Discussion

Synthesis of styrene building blocks: For the postsynthetic modification of DNA strands via the nitrile oxide click reaction we chose two different strategies. For the synthesis of short oligodeoxynucleotides (ODNs) we prepared the styrene phosphoramidite **1** and used it as a building block for standard DNA solid-phase synthesis (Scheme 2). In order to generate long gene fragments with hundreds of styrene units, we prepared the styrene-modified deoxyuridine triphosphate **2**, which we incorporated using PCR. In the PCR reaction TTP is simply replaced by compound **2**.

The synthesis of compound **1** (Scheme 2a) starts with TBDMS protection of 5-iodouridine **3** (yield of 90%) followed by Sonogashira coupling of the TBDMS-protected nucleoside with propargylic alcohol to yield compound **4** in



Scheme 1. Schematic depiction of the postsynthetic functionalization of DNA strands using Cu^I-catalyzed alkyne–azide and Cu-free styrene–nitrile oxide click chemistry. (dATP: deoxyadenosine triphosphate; dCTP: deoxycytidine triphosphate; dGTP: deoxyguanosine triphosphate.).



Scheme 2. a) Synthesis of styrene-bearing phosphoramidite **1** for DNA solid-phase synthesis; b) styrene triphosphate **2** and alkyne triphosphate **7** as building blocks for PCR, and c) list of the prepared oligonucleotides (ODNs) containing nucleoside **5**.[21]

75% yield.[20] Subsequent Williamson ether synthesis with 4-vinylbenzylchloride and TBAF deprotection furnished the styrene-bearing nucleoside **5** in 52% yield over two steps. For DNA solid-phase synthesis the phosphoramidite building block **1** was prepared. DMT protection using standard methods was possible in 85% yield. Final phosphitylation with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite gave compound **1** in 77% yield.[21] Incorporation of the styrene-phosphoramidite **1** into ODNs-**1**–**4** was performed using ultramild DNA solid-phase synthesis (see Scheme 2c).[21,22] Excellent oligonucleotide syntheses were obtained, even in the case of ODN-**4** where six phosphoramidites **1** were coupled in a row. For the PCR-based approach we converted the styrene nucleoside **5** into the corresponding styrene triphosphate **2** (Scheme 2b).[21]

Primer extension studies and RT-PCR investigations: To explore the ability of triphosphate **2** to function as a substrate for DNA polymerases, primer extension studies were performed (Figure 1).[21] The experiment, designed by Held et al.,[23] was carried out with three different templates (**T1**–**T3)** and various polymerases, including the family B polymerases *Pyrococcus woesi* (Pwo) and *Thermococcus litoralis* (Deep vent *exo*$^-$) as well as *Thermococcus kodakaraensis* (KOD XL). For template **T1** only one modified uridine needs to be incorporated into the primer. Copying of **T2** and **T3** requires that the polymerase inserts two and eight modified nucleosides. Elongation of the primer on template **T3** is particularly difficult because it requires reading through a stretch of three consecutive modified bases. All primer extensions were performed with a 5′-fluorescein-labeled primer **P** at 72 °C for 10 min or 1 h (see Figure 1).[21]

The primer extension studies with styrene triphosphate **2** revealed efficient incorporation and full elongation of the primer on all three templates **T1**–**T3** within 10 min reaction time with Pwo and KOD XL polymerase. Even **T3** was efficiently used as a template. Full elongation by Deep vent *exo*$^-$ was only achieved for **T1** and **T2**.

Encouraged by these results, we started to incorporate styrene-triphosphate **2** into longer DNA strands. For this we utilized a real-time (RT) PCR assay with SYBR Green II as fluorescent DNA intercalator. Cycling times, annealing temperatures, chemical additives, and substrate concentrations were carefully screened. We first investigated the ability of the polymerases Pwo, Deep Vent *exo*$^-$ and KOD XL to amplify a 300 base pair (bp) fragment and a 900 bp fragment of the Pol η gene. The 300 bp PCR amplicon features 154 thymidines, which need to be replaced during the PCR. The 900 bp PCR fragment contains 551 thymidines that need to be exchanged. We discovered that this is possible with the KOD XL polymerase, which accepts the triphosphate **2** as a substrate without problems.[21]

Figure 2a shows the 300 bp PCR products PCR300·**N** (dATP, dCTP, TTP, dGTP) and PCR300·**2** (dATP, dCTP, dGTP, **2**). Figure 2c shows the results obtained for the 900 bp fragments PCR900·**N** and PCR900·**2**. Both fragments PCR300·**2** and PCR900·**2** exhibit a reduced mobility on the agarose gels. In Figure 2b and d the corresponding fluorescence curves of the RT-PCR studies are presented. These are similar to typical PCR profiles. The fluorescence signals of the PCR reaction producing PCR300·**2** and PCR900·**2** increase slower in comparison to the unmodified PCR products. This is likely caused by slower incorporation of the triphosphate **2** compared to the natural triphosphate TTP.

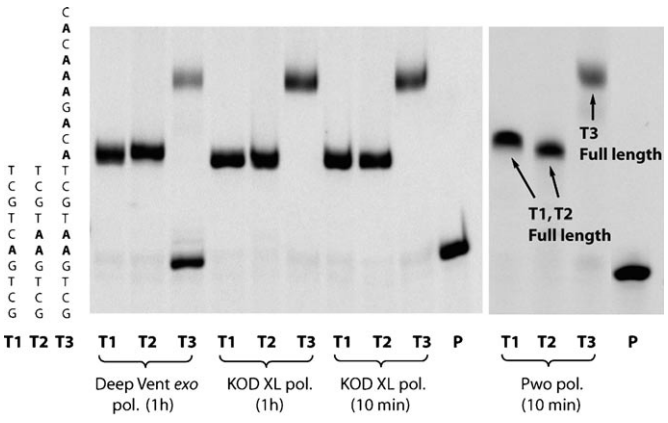


Figure 1. Primer extension experiments with dATP, dCTP, dGTP and dU*TP **2** and the indicated DNA polymerases. Reaction times were either 10 min or 1 h. **T1**–**T3** are the DNA templates and **P** is the 5′-fluorescein-labeled primer (Sequence: 5′-Fluo-GGCTTCTTCGAACCGGG-TAC-3′).[21]

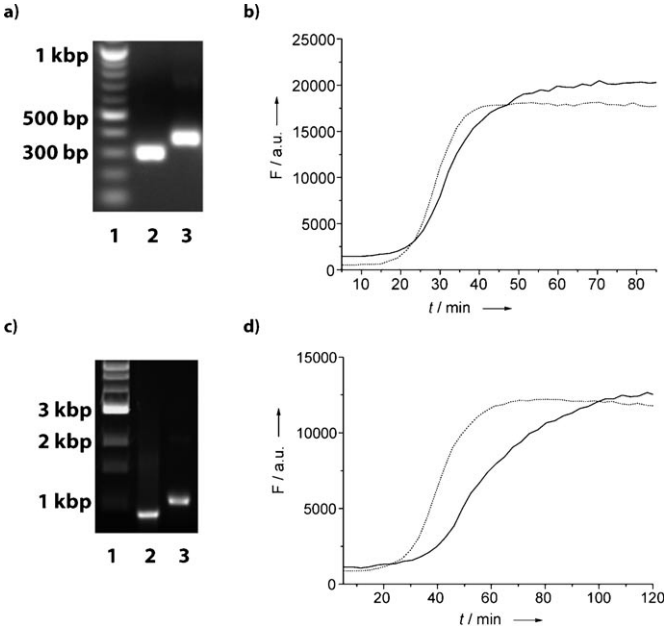
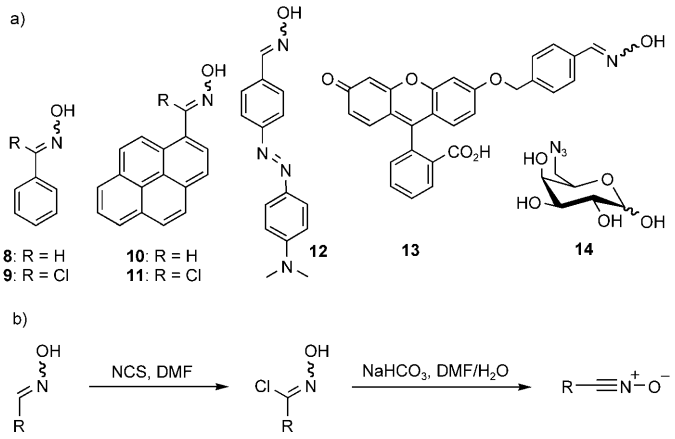


Figure 2. Incorporation of dU*TP **2** into a 300 bp PCR fragment using the KOD XL polymerase. a) Agarose gel of 300 bp fragments; lane 1: DNA ladder; lane 2: dATP, dCTP, dGTP, TTP; lane 3: dATP, dCTP, dGTP, **2**; b) corresponding RT-PCR profiles of the 300 bp amplicons with KOD XL (dashed line: natural triphosphates; solid line: dATP, dCTP, dGTP, **2**); c) agarose gel of 900 bp fragments; lane 1: DNA ladder; lane 2: dATP, dCTP, dGTP, TTP; lane 3: dATP, dCTP, dGTP, **2**; d) additional RT-PCR profiles of the 900 bp PCR fragments (dashed line: natural triphosphates; solid line: dATP, dCTP, dGTP, **2**).[21]

Cycloaddition of nitrile oxides to short styrene-modified ODNs: For the cycloaddition reaction of the styrene-modified DNA strands with the nitrile oxides derived from **8**–**13** we either mixed *N*-chlorosuccinimide (NCS) and the aldoxime (**8**, **10**, **12** or **13**) in the presence of a weak base to produce the corresponding hydroximoyl chloride in situ (Huisgen's in situ method)[24] or we directly used the hydroximoyl chloride (**9** or **11**) without any base present during the reaction. The concentration of the nitrile oxide was found to be even under these neutral conditions high enough to allow efficient reaction (see Scheme 3b).[21] The nitrile oxide solution was added directly to the solid supported ODNs-**1**–**4** (Scheme 2c) or to an unbuffered solution containing the purified, deprotected ODNs-**1**–**4**.[21]



Scheme 3. a) Nitrile oxide precursors **8**–**13** as well as azide **14**, and b) nitrile oxide preparation via hydroximoyl chlorides.[21]

We initially performed the click reaction on solid supported ODN-**1** comprising one styrene functionality. This material was reacted with the nitrile oxide precursors **9** and **11** in pure water. Reaction with **12** and **13** required first addition of NCS to the precursors. This mixture was added to the supported DNA with some base ($NaHCO_3$). 20-fold excess of the nitrile oxides was allowed to react with the DNA by shaking the reaction mixture for 1 h at room temperature.[21] ODN-**2** containing two styrene moieties was reacted with nitrile oxide precursor **9** directly in the DNA synthesizer. For reaction in the synthesizer we simply pumped the nitrile oxide solution through the DNA cartridge containing solid-supported ODN-**2** and washed after 20 min thoroughly with acetonitrile.[21] The CPG material was washed afterwards with DMF as well as with CH_2Cl_2 and finally dried. The labeled DNA was cleaved from the solid support with potassium carbonate (50 mM in MeOH) over 3 h at room temperature and purified by reversed-phase HPLC.[21]

Figure 3 shows representatively the MALDI-TOF and HPLC data of the unpurified click product ODN-**1a** (ODN-**1** + **8**, Figure 3a), ODN-**1c** (ODN-**1** + **12**, Figure 3b), and ODN-**2a** (ODN-**2** + **9**, Figure 3c). All nitrile oxides depicted in Scheme 3 were found to react quantitatively. The starting DNA material was in all cases completely transformed. The corresponding MALDI-TOF spectra are in full agreement with the expected molecular weights for the DNA products. Significantly, we detected no by-products, which showed us that the conversion proceeded cleanly in all cases on all ODNs under the various conditions.

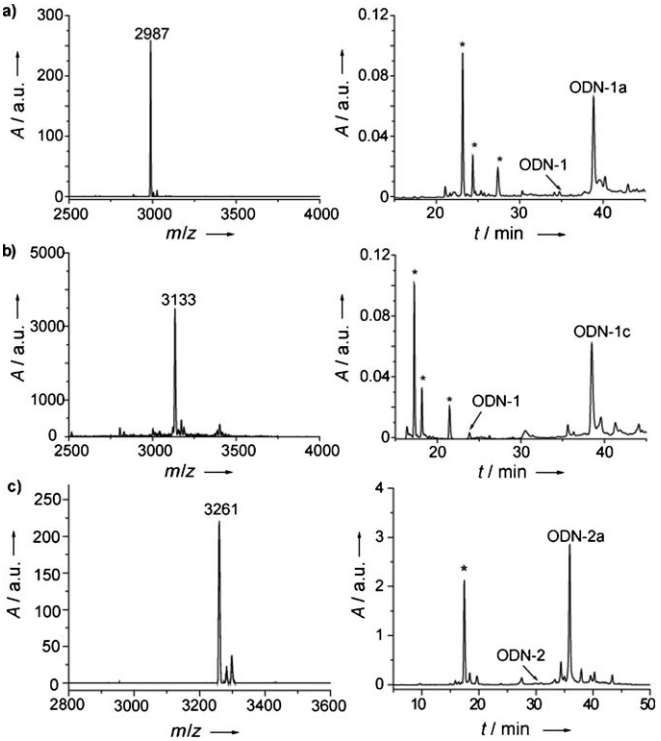


Figure 3. Raw MALDI-TOF spectra and HPLC traces of a) the unpurified click reaction performed with ODN-**1** (M_W 2868) containing one styrene moiety and nitrile oxide precursor **8** (ODN-**1a**, M_W 2987) on solid support; b) the raw click product ODN-**1c** (M_W 3134) produced with solid supported ODN-**1** (M_W 2868) and dabcyl nitrile oxide precursor **12**; c) the crude click product obtained by click reaction of ODN-**2** (M_W 3021) comprising of two consecutive styrene moieties and nitrile oxide precursor **9** (ODN-**2a**, M_W 3259) directly in the DNA synthesizer.[21] (* = by-products of the DNA synthesis.)

We also performed the nitrile oxide cycloaddition with the styrene modified short ODNs in solution after DNA purification. For these experiments we exclusively used hydroximoyl chlorides as precursors for the nitrile oxide solution without any base and utilized pure water as the solvent (see Figures S2–S5).

The same clean conversions were also observed for ODN-**4** comprising six consecutive styrene units. Reaction of ODN-**4** with nitrile oxide precursor **9** furnished one clean reaction product which could undoubtedly be assigned to the six-fold reacted ODN-**4** (see Figure S4).[21]

Analysis of click efficiency: Investigation of the click transformation was performed by enzymatic digestions of the various DNA reaction products.[21] Enzymatic digests were obtained by using a mixture of DNA degrading enzymes (*Cro-*

talus adamanteus snake venom phosphodiesterase I and alkaline phosphatase; see Supporting Information). The obtained nucleoside mixtures were subsequently analyzed by HPLC-MS.[21] The results are depicted in Figure 4. Enzymatic digests of ODN-**1** (Figure 4a) before the click reaction reveals the canonical bases and the styrene nucleoside **5** (retention time = 58 min). We found that the digest around the modified nucleoside is not complete. In most cases we obtained the modified nucleoside, for example **5**, and the dinucleotide, for example **5**+T. This dinucleotide furnishes the additional peak with a retention time of 48 min. HPLC-MS confirmed that this peak is caused by dinucleotide **5**+T (see also Tables S6 and S7). The enzyme mixtures seem to have problems cleaving the linking phosphodiester bond.[21]

Further HPLC analysis of the enzymatic digestions of the reaction products ODN-**1a** (Figure 4b), ODN-**1b** (Figure 4c), and ODN-**1c** (Figure 4d) showed beside the canonical DNA bases only the corresponding phenyl-modified

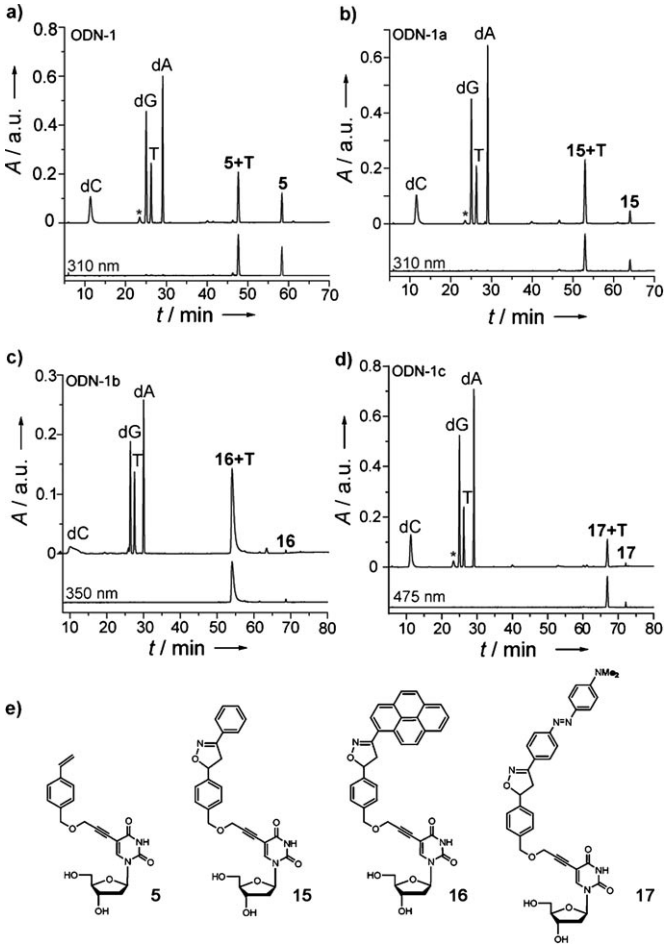


Figure 4. HPLC analysis of the enzymatic digestions of the nitrile oxide modified oligonucleotide ODN-**1**. a) ODN-**1** prior to click reaction. b) ODN-**1** reacted with nitrile oxide precursor **8** (ODN-**1a**) containing **15**, c) ODN-**1** after click reaction with nitrile oxide precursor **10** (ODN-**1b**) containing **16**, and d) ODN-**1** reacted with nitrile oxide precursor **12** (ODN-**1c**) containing **17**. The additional traces show the HPLC data at the diagnostic wavelengths $\lambda = 310$, 350, or 475 nm. (* = inosine formed due to deamination of dA during the assay.)

DNA base **15** (retention time = 63 min), pyrene-derivative **16** (retention time = 67 min), or dabcyl-functionalized nucleoside **17** (retention time = 72 min). The HPLC trace at the diagnostic wavelength of $\lambda = 310$ nm for C5-modified pyrimidines proved for all digests full conversion as no styrene nucleoside **5** was detected anymore. Additional support for the completeness of the click transformation was gained from HPLC chromatograms with a detection wavelength of $\lambda = 350$ nm for the pyrene system (Figure 4c) and $\lambda = 475$ nm for the dabcyl-dye (Figure 4d). In both cases only one product peak appeared. Most important, independent of the detection wavelength no side reactions of the nitrile oxides with, for example, the canonical bases were observed.

Click modifications of PCR products: We next investigated the click reaction on styrene modified PCR300·**2**, obtained with triphosphate **2**. This PCR fragment was reacted with the nitrile oxide precursor **9**. The results of the high density functionalization experiments were again analyzed by enzymatic digestion (different protocol, see Supporting Information) after purification of the product DNA strands via simple ethanol precipitation (see Figure 5).[21]

The HPLC-MS analysis of the digest of the PCR300·**2** starting material (Figure 5a) showed the four canonical DNA bases (T arising from the unmodified primer strands needed for PCR) and the styrene nucleoside **5** (retention time = 58 min). Analysis of the amplicon PCR300·**2a** (Figure 5b) after click reaction with benzonitrile oxide, ethanol precipitation and enzymatic digestion showed the four natural DNA bases and the corresponding 5-phenyl-2-isoxazoline derivative **15** (retention time = 63 min). Surprisingly, no styrene nucleoside **5** (retention time = 58 min) was detected even though every PCR300·**2** molecule contains 154 styrene modifications. These were obviously all converted into the corresponding cycloaddition products, which proves the amazing efficiency of this mild click reaction (see also Figure S6 and Tables S6–S7).

We next investigated the possibility to combine the nitrile oxide based modification of styrene containing oligonucleotides with the Cu^{I}-catalyzed alkyne–azide click reaction. To this end we synthesized a 300 bp PCR fragment by using a mixture of the styrene triphosphate **2** and the alkyne triphosphate **7** (**2**/**7** 1:1).[21] In this experiments the 154 thymidines are exchanged by alkyne and styrene carrying thymidine derivatives in a statistical fashion. Figure 5c shows the enzymatic digestion data of PCR300·**2/7**. Indeed, this amplicon contains next to the canonical bases the styrene building block **5** (retention time = 58 min) and the alkyne nucleoside **6** (retention time = 26 min), which proves the successful incorporation of both modified triphosphates.[21]

To introduce two different labels we first performed the Cu-catalyzed click reaction with the sugar azide **14**. After click reaction we purified the sugar-modified PCR product via ethanol precipitation. We subsequently performed the nitrile oxide click reaction using the nitrile oxide precursor **9**. The final amplicon reaction product was isolated by isopropanol precipitation. After intensive washing PCR300·**2a/**

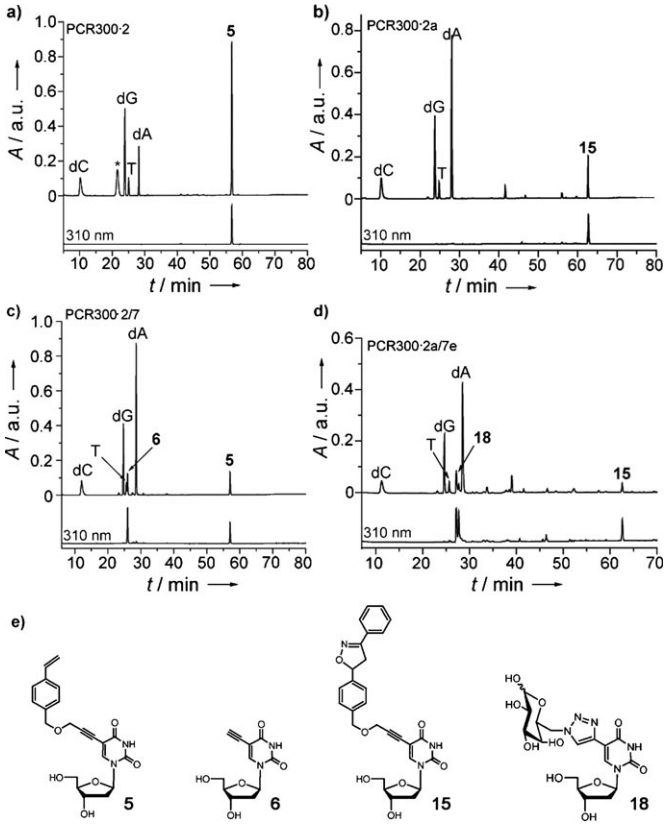


Figure 5. HPLC data of enzymatic digests of the PCR-product PCR300·**2** a) before and b) after the click reaction with **9** to the corresponding product PCR300·**2a**; c) HPLC traces of the enzymatic digestions of the PCR-fragment PCR300·**2/7** c) before and d) after the Cu^{I}-catalyzed click-reaction with azide **14** and the Cu-free click reaction with benzonitrile oxide precursor **9** to the corresponding product PCR300·**2a/7e**, and e) depiction of the detected nucleosides. The nucleobase T comes from the unmodified primers that were used for the PCR experiment.

7e was subjected to analysis by enzymatic digest. Figure 5d shows the results obtained for the double labeled PCR300·**2a/7e** fragment: Next to the canonical DNA bases only the double peaks of the sugar click product **18** (retention times = 27.1 and 27.7 min) and of the 5-phenyl-2-isoxazoline nucleobase **15** (retention time = 63 min) were detected. The clean reaction is nicely proven at the detection wavelength of $\lambda = 310$ nm, which shows only three main products. The sugar nucleoside gives two signals because of rapid anomerization of the sugar moiety. The obtained result is in two respects remarkable. First, because the two click reactions are truly orthogonal. The azide reaction proceeds smoothly at the alkynes, leaving the styrene units intact. More important is, however, the observation that no unreacted alkyne or styrene building block was detected, showing that both reactions are extremely high yielding even on complex oligonucleotide substrates. One should keep in mind that a 300 bp DNA fragment has a molecular weight of approximately 90 kDa, which is in the range of a normal size protein. Chemistry that enables the quantitative conversion of 154 reaction sites on such complex chemical entities, which are surely folded in aqueous solution, has the potential to change the way of how modified oligonucleotides are prepared today. Further LC-ESI-MS measurements listed in the Supporting Information support this vision (Figure S8, Table S8).[21]

Conclusion

We have shown here that the 1,3-dipolar cycloaddition of nitrile oxides with styrene modified oligodeoxynucleotides (9 bp) and long PCR amplicons (300 bp) is an extremely facile and efficient approach for the high density functionalization of DNA. This mild click reaction on styrene-bearing DNA gives exclusively the corresponding 3,5-disubstituted isoxazoline cycloproducts. The reaction is easy to perform on solid support, directly in the DNA synthesizer or in solution. The ready availability of all starting materials, short reaction times and the rather simple procedures involved provide the DNA material in excellent quantity and quality. The chemistry is orthogonal to the Cu^{I}-catalyzed reaction of alkyne-modified DNA with azide-carrying molecules allowing the sequential labeling of alkyne- and styrene-modified PCR amplicons.

Acknowledgements

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[1] a) N. C. Seeman, *Nature* **2003**, *421*, 427–431; b) N. C. Seeman, *Mol. Biotechnol.* **2007**, *37*, 246–257; c) U. Feldkamp, C. M. Niemeyer, *Angew. Chem.* **2006**, *118*, 1888–1910; *Angew. Chem. Int. Ed.* **2006**, *45*, 1856–1876; d) M. Endo, H. Sugiyama, *ChemBioChem* **2009**, *10*, 2420–2443.

[2] D. A. Rusling, G. Peng, N. Srinivasan, K. R. Fox, T. Brown, *Nucleic Acids Res.* **2008**, *37*, 1288–1296.

[3] a) M. K. Beissenhirtz, I. Willner, *Org. Biomol. Chem.* **2006**, *4*, 3392–3401; b) I. Willner, B. Willner, E. Katz, *Bioelectrochemistry* **2007**, *70*, 2–11; c) F. A. Aldaye, A. L. Palmer, H. F. Sleiman, *Science* **2008**, *321*, 1795–1799; d) A. Heckel, M. Famulok, *Biochimie* **2008**, *90*, 1096–1107; e) C. M. Niemeyer, *Angew. Chem.* **2010**, *122*, 1220–1238; *Angew. Chem. Int. Ed.* **2010**, *49*, 1200–1216; f) L. Xu, D. Liu, *Chem. Soc. Rev.* **2010**, *39*, 150–155; g) L. Röglin, O. Seitz, *Org. Biomol. Chem.* **2008**, *6*, 3881–3887; h) J. S. Hartig, S. H. Najafi-Shoushtari, I. Grune, A. Yan, A. D. Ellington, M. Famulok, *Nat. Biotechnol.* **2002**, *20*, 717–722; i) M. Fischler, A. Sologubenko, J. Mayer, G. Clever, G. Burley, J. Gierlich, T. Carell, U. Simon, *Chem. Commun.* **2008**, 169–171; j) J. Elbaz, R. Tel-Vered, R. Freeman, H. B. Yildiz, I. Willner, *Angew. Chem.* **2009**, *121*, 139–143; *Angew. Chem. Int. Ed.* **2009**, *48*, 133–137; k) C. T. Wirges, J. Timper, M. Fischler, A. S. Sologubenko, J. Mayer, U. Simon, T. Carell, *Angew. Chem.* **2009**, *121*, 225–229; *Angew. Chem. Int. Ed.* **2009**, *48*, 219–223.

[4] a) G. Mayer, D. Ackermann, N. Kuhn, M. Famulok, *Angew. Chem.* **2008**, *120*, 985–987; *Angew. Chem. Int. Ed.* **2008**, *47*, 971–973; b) K. Borjesson, S. Preus, A. H. El-Sagheer, T. Brown, B. Albinsson, L. M. Wilhelmsson, *J. Am. Chem. Soc.* **2009**, *131*, 4288–4293; c) V. Raindlová, R. Pohl, M. Šanda, M. Hocek, *Angew. Chem.* **2010**, *122*, 1082–1084; *Angew. Chem. Int. Ed.* **2010**, *48*, 1064–1066; d) H.

Cahová, L. Havran, P. Brazdilova, H. Pivonkova, R. Pohl, M. Fojta, M. Hocek, *Angew. Chem.* **2008**, *120*, 2089–2092; *Angew. Chem. Int. Ed.* **2008**, *47*, 2059–2062; e) M. Vrabel, P. Horakova, H. Pivonkova, L. Kalachova, H. Cernocka, H. Cahova, R. Pohl, P. Sebest, L. Havran, M. Hocek, M. Fojta, *Chem. Eur. J.* **2009**, *15*, 1144–1154.

[5] a) G. H. Clever, C. Kaul, T. Carell, *Angew. Chem.* **2007**, *119*, 6340–6350; *Angew. Chem. Int. Ed.* **2007**, *46*, 6226–6236; b) J. Müller, *Eur. J. Inorg. Chem.* **2008**, 3749–3763; c) K. Tanaka, G. H. Clever, Y. Takezawa, Y. Yamada, C. Kaul, M. Shionoya, T. Carell, *Nat. Nanotechnol.* **2006**, *1*, 190–194; d) H. Yang, C. K. McLaughlin, F. A. Aldaye, G. D. Hamblin, A. Z. Rys, I. Rouiller, H. F. Sleiman, *Nat. Chem. Biol.* **2009**, *5*, 390–396; e) S. Johannsen, N. Megger, D. Böhme, R. K. Sigel, J. Müller, *Nat. Chem.* **2010**, *2*, 229–234.

[6] S. H. Weisbrod, A. Marx, *Chem. Commun.* **2008**, 5675–5685.

[7] a) R. Huisgen, *Angew. Chem.* **1963**, *75*, 604–637; *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 565–632; b) R. Huisgen, G. Szeimies, L. Möbius, *Chem. Ber.* **1967**, *100*, 2494–2507; c) V. Hong, S. I. Presolski, C. Ma, M. G. Finn, *Angew. Chem.* **2009**, *121*, 10063–10067; *Angew. Chem. Int. Ed.* **2009**, *48*, 9879–9883.

[8] C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.

[9] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.

[10] P. M. E. Gramlich, C. T. Wirges, A. Manetto, T. Carell, *Angew. Chem.* **2008**, *120*, 8478–8487; *Angew. Chem. Int. Ed.* **2008**, *47*, 8350–8358.

[11] a) G. A. Burley, J. Gierlich, M. R. Mofid, H. Nir, S. Tal, Y. Eichen, T. Carell, *J. Am. Chem. Soc.* **2006**, *128*, 1398–1399; b) J. Gierlich, G. A. Burley, P. M. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639–3642; c) J. Gierlich, K. Gutsmiedl, P. M. Gramlich, A. Schmidt, G. A. Burley, T. Carell, *Chem. Eur. J.* **2007**, *13*, 9486–9494; d) P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem.* **2008**, *120*, 3491–3493; *Angew. Chem. Int. Ed.* **2008**, *47*, 3442–3444; e) F. Seela, V. R. Sirivolu, *Org. Biomol. Chem.* **2008**, *6*, 1674–1687; f) V. R. Sirivolu, P. Chittepu, F. Seela, *ChemBioChem* **2008**, *9*, 2305–2316; g) F. Seela, H. Xiong, P. Leonard, S. Budow, *Org. Biomol. Chem.* **2009**, *7*, 1374–1387.

[12] a) S. Thyagarajan, N. N. Murthy, A. A. Narducci Sarjeant, K. D. Karlin, S. E. Rokita, *J. Am. Chem. Soc.* **2006**, *128*, 7003–7008; b) F. Wolbers, P. ter Braak, S. Le Gac, R. Luttge, H. Andersson, I. Vermes, A. van den Berg, *Electrophoresis* **2006**, *27*, 5073–5080.

[13] a) N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047; b) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793–16797; c) S. T. Laughlin, J. M. Baskin, S. L. Amacher, C. R. Bertozzi, *Science* **2008**, *320*, 664–667; d) E. M. Sletten, C. R. Bertozzi, *Angew. Chem.* **2009**, *121*, 7108–7133; *Angew. Chem. Int. Ed.* **2009**, *48*, 6974–6998; e) P. V. Chang, J. A. Prescher, E. M. Sletten, J. M. Baskin, I. A. Miller, N. J. Agard, A. Lo, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 1821–1826; f) A. A. Poloukhtine, N. E. Mbua, M. A. Wolfert, G. J. Boons, V. V. Popik, *J. Am. Chem. Soc.* **2009**, *131*, 15769–15776; g) X. Ning, J. Guo, M. A. Wolfert, G. J. Boons, *Angew. Chem.* **2008**, *120*, 2285–2287; *Angew. Chem. Int. Ed.* **2008**, *47*, 2253–2255; h) M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. Rutjes, J. C. van Hest, F. L. van Delft, *Chem. Commun.* **2010**, 97–99.

[14] a) M. L. Blackman, M. Royzen, J. M. Fox, *J. Am. Chem. Soc.* **2008**, *130*, 13518–13519; b) N. K. Devaraj, R. Weissleder, S. A. Hilderbrand, *Bioconjugate Chem.* **2008**, *19*, 2297–2299; c) N. K. Devaraj, R. Upadhyay, J. B. Haun, S. A. Hilderbrand, R. Weissleder, *Angew. Chem.* **2009**, *121*, 7147–7150; *Angew. Chem. Int. Ed.* **2009**, *48*, 7013–7016.

[15] a) I. Singh, J. S. Vyle, F. Heaney, *Chem. Commun.* **2009**, 3276–3278; b) V. Algay, I. Singh, F. Heaney, *Org. Biomol. Chem.* **2010**, *8*, 391–397; c) I. Singh, F. Heaney, *Org. Biomol. Chem.* **2010**, *8*, 451–456.

[16] a) S. S. van Berkel, A. T. Dirks, M. F. Debets, F. L. van Delft, J. J. Cornelissen, R. J. M. Nolte, F. P. Rutjes, *ChemBioChem* **2007**, *8*, 1504–1508; b) S. S. van Berkel, A. T. Dirks, S. A. Meeuwissen, D. L. Pingen, O. C. Boerman, P. Laverman, F. L. van Delft, J. J. Cornelissen, F. P. Rutjes, *ChemBioChem* **2008**, *9*, 1805–1815.

[17] a) W. Song, Y. Wang, J. Qu, M. M. Madden, Q. Lin, *Angew. Chem.* **2008**, *120*, 2874–2877; *Angew. Chem. Int. Ed.* **2008**, *47*, 2832–2835; b) W. Song, Y. Wang, J. Qu, Q. Lin, *J. Am. Chem. Soc.* **2008**, *130*, 9654–9655; c) Y. Wang, W. Song, W. J. Hu, Q. Lin, *Angew. Chem.* **2009**, *121*, 5434–5437; *Angew. Chem. Int. Ed.* **2009**, *48*, 5330–5333.

[18] K. Gutsmiedl, C. T. Wirges, V. Ehmke, T. Carell, *Org. Lett.* **2009**, *11*, 2405–2408.

[19] a) M. D. Best, *Biochemistry* **2009**, *48*, 6571–6584; b) P. Kele, G. Mezo, D. Achatz, O. S. Wolfbeis, *Angew. Chem.* **2009**, *121*, 350–353; *Angew. Chem. Int. Ed.* **2009**, *48*, 344–347; c) P. Kele, X. Li, M. Link, K. Nagy, A. Herner, K. Lorincz, S. Beni, O. S. Wolfbeis, *Org. Biomol. Chem.* **2009**, *7*, 3486–3490.

[20] M. Kuwahara, J. Nagashima, M. Hasegawa, T. Tamura, R. Kitagata, K. Hanawa, S. Hososhima, T. Kasamatsu, H. Ozaki, H. Sawai, *Nucleic Acids Res.* **2006**, *34*, 5383–5394.

[21] See the Supporting Information.

[22] B. Horten, *Nature* **1998**, *396*, 391–392.

[23] H. A. Held, S. A. Benner, *Nucleic Acids Res.* **2002**, *30*, 3857–3869.

[24] W. Fliege, R. Huisgen, *Liebigs Ann. Chem.* **1973**, 2038–2047.

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