DNA Click Chemistry

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Postsynthetic DNA Modification through the Copper-Catalyzed Azide–Alkyne Cycloaddition Reaction

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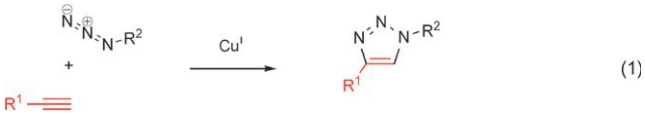
bioconjugation · click chemistry · DNA · polymerase chain reaction · solid-phase synthesis

The attachment of labels onto DNA is of utmost importance in many areas of biomedical research and is valuable in the construction of DNA-based functional nanomaterials. The copper(I)-catalyzed Huisgen cycloaddition of azides and alkynes (CuAAC) has recently been added to the repertoire of DNA labeling methods, thus allowing the virtually unlimited functionalization of both small synthetic oligonucleotides and large gene fragments with unprecedented efficiency. The CuAAC reaction yields the labeled polynucleotides in very high purity after a simple precipitation step. The reviewed technology is currently changing the way in which functionalized DNA strands are generated cost-efficiently in high quality for their application in molecular diagnostics systems and nanotechnological research.

1. Introduction

The 1,3-dipolar cycloaddition reaction of azides and alkynes—the Huisgen reaction[1]—has recently found a tremendous number of novel applications[2] (ca. 1000 publications) after the discovery that it can be efficiently catalyzed by copper(I) [Eq. (1)].[3] The multistep copper-catalyzed azide–alkyne cycloaddition (CuAAC), which is reviewed herein, is presently the most prominent example of a group of reactions named click reactions.[4] According to Sharpless' definition, these reactions are characterized by high yields, mild reaction conditions, and by their tolerance of a broad range of functional groups. Typically, the reactions require simple or no workup or purification of the product.



(1)

The most important characteristic of the CuAAC reaction is its unique bioorthogonality, as neither azide nor terminal alkyne functional groups are generally present in natural systems. A wide range of biomolecules has thus been labeled to date, including peptides,[5] proteins,[6] polysaccharides,[7] and even entire viruses[8] and cells.[9] The use of this method for DNA modification has been somewhat delayed by the fact that copper ions damage DNA, typically yielding strand breaks.[10] As these problems have now been overcome by the use of the copper(I)-stabilizing ligand tris(benzyltriazolylmethyl)amine (TBTA),[11] the CuAAC reaction is in an excellent position to take over as the state-of-the-art methodology to label and modify DNA.

In a variety of applications, such as molecular diagnostics, DNA needs to bear labels (e.g. fluorescent dyes) for detection of the DNA molecule. Moreover, biotin is often attached to DNA to facilitate DNA purification, or most recently, carbohydrates have been used to functionalize DNA to allow selective silver deposition or to set up carbohydrate microarrays. Two general strategies exist for the labeling of oligonucleotides, called presynthetic and postsynthetic labeling (Figure 1). Presynthetic labeling denotes that the nucleotide monomers already carry the desired label before DNA synthesis, deprotection, and purification. That is, these modified oligonucleotides are incorporated into the DNA strand during the usual phosphoramidite process. Postsynthetic labeling requires the introduction of a small reactive

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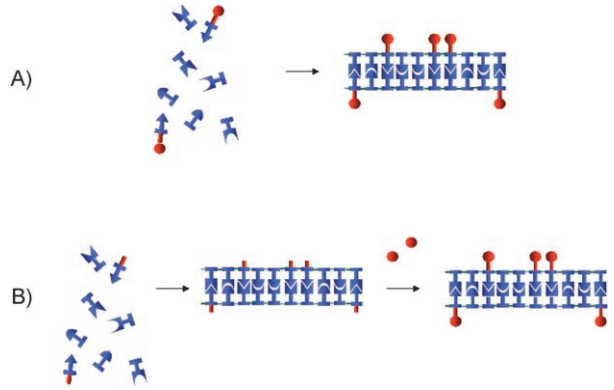

Figure 1. Schematic representation for presynthetic (A; blue: nucleotides, red: markers) and postsynthetic labeling (B; blue: nucleotides, red sticks: reactive groups, red balls: markers).

moiety into DNA, which can be coupled to the label after completion of DNA synthesis.

In the presynthetic strategy (Figure 1 A) a phosphoramidite bearing the desired label is synthesized, which needs to be stable enough to survive the harsh conditions employed during solid-phase synthesis (acidic, alkaline, and oxidative conditions) and deprotection (strongly alkaline conditions) of the DNA strand. Additionally, the phosphorus(III) atom of the phosphoramidite group, which is susceptible to oxidation to phosphorus(V) in the corresponding phosphoramidate, needs to be stable in the presence of the label. This method thus excludes the use of labels with reactive functional groups or inherent base- or acid-sensitivity. Alternatively, the label can be prepared as the corresponding triphosphate, in which case DNA polymerases are used to insert the label during primer extension or polymerase chain reaction (PCR). This method is limited to those modifications which are accepted as substrates by polymerases, and there are often steric limitations.

In the postsynthetic labeling strategy (Figure 1 B), a small reactive group is introduced into DNA, which can then be conjugated to the desired functional molecule in a selective manner after DNA synthesis and deprotection. Presently, the most widely utilized method for postsynthetic labeling is to prepare DNA bearing an amino group (amino modifiers), which reacts with an active ester functional group (e.g. *N*-hydroxysuccinimide units) of the desired modification.[12] Sensitive or reactive moieties can be introduced in this way, but the strategy is highly dependent on the selectivity and efficiency of the postsynthetic modification step. This strategy allows for a higher degree of modularity, because changing the label does not require a novel nucleotide synthesis, as in the case with presynthetic labeling. However, the low coupling yields and the need for intensive oligonucleotide purification by HPLC after labeling limit this approach and make labeled oligonucleotides very expensive compounds. Nucleobases that carry the reactive amino group can also be used as triphosphates for the incorporation into DNA by either primer extension or PCR.[13]

The CuAAC reaction offers solutions to the issues addressed above. The chemistry is clean and high-yielding and operates at mild conditions. To allow oligonucleotide modification by this reaction, however, DNA building blocks are required which carry either alkyne or azide functional groups. As depicted in Scheme 1, alkyne or azide functionalities have already been attached to many different positions on DNA. Moreover, a number of non-nucleosidic alkyne or azide DNA modifiers have been prepared.[14,15] It must be

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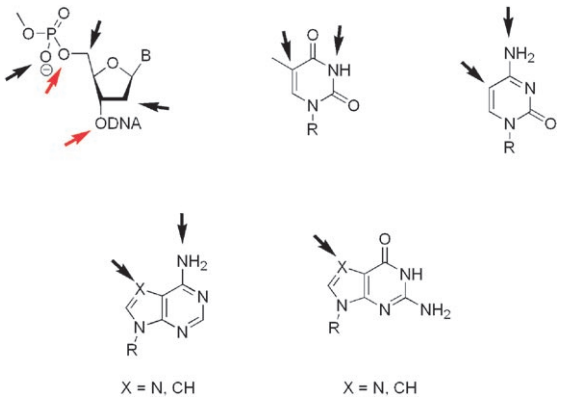


Scheme 1. Overview over the different positions that have been used for the attachment of azides or alkynes to DNA. Red arrows indicate attachment points for terminal azide and alkyne residues only. B = nucleobase

noted, however, that the synthesis of azide-modified DNA by solid-phase synthesis is intrinsically difficult, as azides are reduced by the phosphorus(III) atom of the phosphoramidites.[16] This constitutes a limitation of the CuAAC approach to DNA labeling, as azide-modified DNA is not directly accessible by solid-phase DNA synthesis.

In this Minireview, we summarize the various approaches to modify oligonucleotides using the CuAAC reaction. In Sections 2–6 we describe applications of DNA strands prepared by solid-phase synthesis and modification using the CuAAC reaction.[16] In Section 7, we summarize click-chemistry approaches in combination with enzymatic strategies.

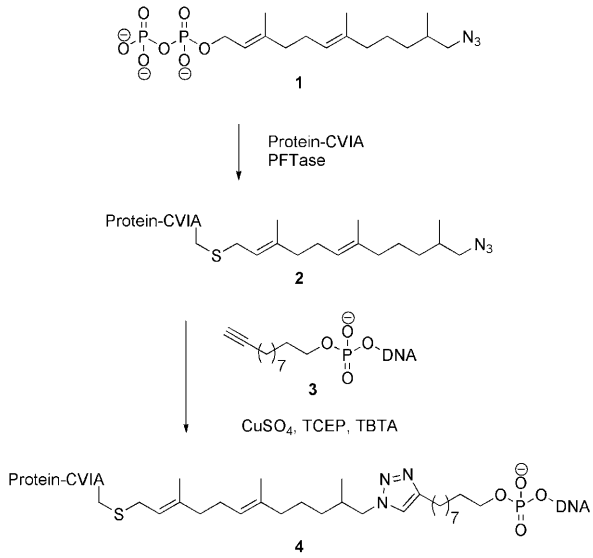
2. Preparation of Surface-Immobilized DNA

The first example of a successful modification of DNA[*] using Huisgen azide–alkyne cycloaddition used a 5′-alkylamino-modified DNA strand, which was treated with succinimidyl-5-azidovalerate to display an azide group at the 5′-terminus.[17,18] Thermal (not copper(I)-catalyzed) cycloaddition with an alkyne-modified fluorescein derivative furnished the reaction product in excellent yield, but as a regioisomeric mixture of the 1,4- and 1,5-triazoles. These mixtures are typical for the Huisgen cycloaddition without copper(I) catalysis. The direct incorporation of azides into synthetic DNA strands is, as mentioned above, intrinsically difficult, because the azide group reacts with the phosphorus(III) atom of the phosphoramidite group in a Staudinger reduction.[16] However, a two-step procedure described above for attaching the azide to the DNA strand allowed the subsequent attachment using CuAAC of the DNA to a glass surface for a sequencing-by-synthesis approach.[19] An alternative approach for the immobilization of DNA on surfaces using the CuAAC reaction has employed an alkyne group attached to the 5′-OH group of a DNA strand, which reacted with azide-modified gold surfaces.[20] Even simpler is the approach described by Reinhoudt and co-workers. This group pressed a polydimethylsiloxane (PDMS) stamp, which was covered with alkyne-modified DNA, directly onto an azide-terminated glass slide to achieve the azide–alkyne cycloaddition reaction under pressure without added copper(I) catalyst.[15]

[*] At this point, the cycloaddition was performed thermally without copper(I) catalysis, owing to DNA-degrading effect of free copper(I) species in aqueous solutions. After publication of a copper(I)-stabilizing ligand, which accelerates the click reaction but avoids the presence of copper(I) ions in solution, most subsequent work made use of the copper(I)-catalyzed version of the Huisgen cycloaddition. Nonetheless, the work by Ju and co-workers is seen as the prototypical example of click chemistry on DNA.

3. Preparation of DNA–Protein Conjugates

The CuAAC reaction has been successfully utilized for the preparation of complex DNA–protein conjugates.[21] Distefano and co-workers attached the specific tetrapeptide CVIA to green fluorescent protein (GFP). To this tag the authors specifically attached an azide-modified isoprenoid diphosphate **1** with the help of the enzyme farnesyltransferase (PFTase, Scheme 2).[22] The obtained azide-bearing protein

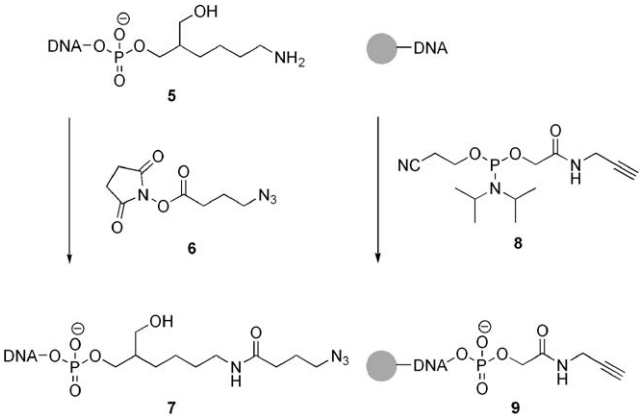


Scheme 2. Synthetic strategy for DNA–protein conjugates. TCEP = tris(2-carboxyethyl)phosphane hydrochloride, TBTA = tris(benzyltriazolylmethyl)amine.

was then treated with a single-stranded oligonucleotide (ssODN) carrying an alkyne attached to a 5′-terminal phosphate group. To test whether the DNA sequence can still be addressed despite the presence of the protein, a counter strand containing a Texas Red label was successfully hybridized to the DNA strand attached to the protein. Using this construct, the group successfully built up nanoscale DNA tetrahedra,[23] which were finally decorated with up to four GFPs. The methodology is currently being exploited for the preparation of protein-modified DNA nanostructures.

4. Preparation of Cyclic and Branched DNA Structures

The CuAAC reaction has vastly simplified the synthesis of cyclic and catenated DNA structures.[16,24] Brown and co-workers have devised a strategy to label a ssODN with both a 5′-terminal alkyne and a 3′-terminal azide.[25] The azide was generated as described by Ju and co-workers[17] by coupling of succinimidyl-4-azidobutyrate to an amino linker at the DNA strand, yielding the required 3′-terminal azide (**7** in Scheme 3). The alkyne group, in contrast, was introduced at the 5′-terminus by terminating the solid-phase synthesis with

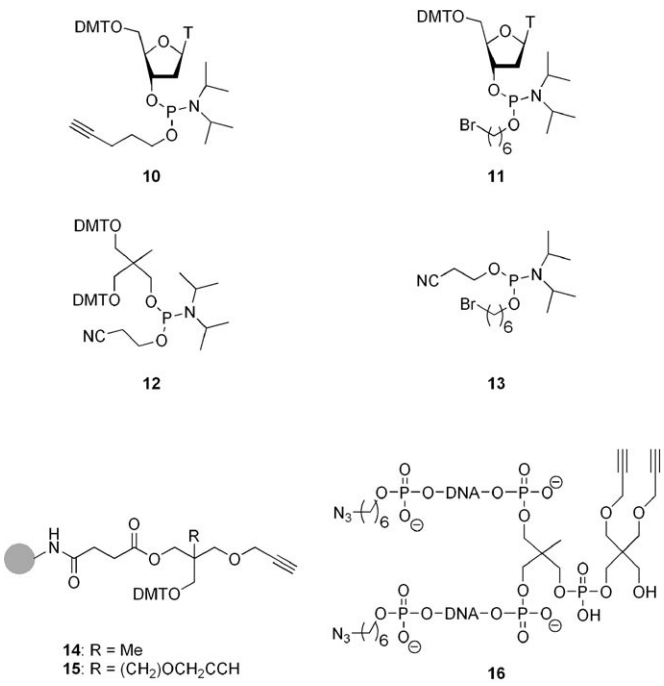


Scheme 3. Building blocks for the synthesis of cyclic and catenated DNA. ● = Solid resin.

the propargylated phosphoramidite **8**. Such a doubly modified DNA strand reacts under high-dilution conditions with itself in a CuAAC reaction to give a cyclic ssODN. When the complementary ODN, which also carries an azide and an alkyne group, is hybridized to this cyclic ODN, it can in turn also be cyclized to yield a covalently catenated double-stranded (ds) DNA. In this case, the first ODN templates the CuAAC reaction of the second DNA strand, which reduces side reactions. The catenated DNA showed a vastly increased resistance to exonuclease degradation.

The exonuclease stability of cyclic DNA strands was also exploited by Matsuda and co-workers.[26] This group prepared a dsODN that binds to the κB transcription factor as a decoy molecule to inhibit in vitro and ex vivo transcription. Then propargyl and azidoethyl linkers on the N3-position of thymidine were introduced. The dangling 5′-azide and 3′-alkyne groups of the dsODN were used to form a circular DNA strand, which can also be viewed as a double hairpin or dumbbell structure. The internal double-stranded binding region of this decoy molecule was not significantly distorted, whereas the thermal stability and the exonuclease stability were strongly increased. The binding affinity to the nuclear factor was shown not to be hampered by the cyclization.

A whole range of different DNA structures was made accessible by the work of Morvan and co-workers.[27] Alkynyl and bromoalkyl groups were introduced at the phosphoramidite group of nucleotides **10** and **11**, respectively, to allow for labeling inside a DNA strand (Scheme 4). The bromoalkyl



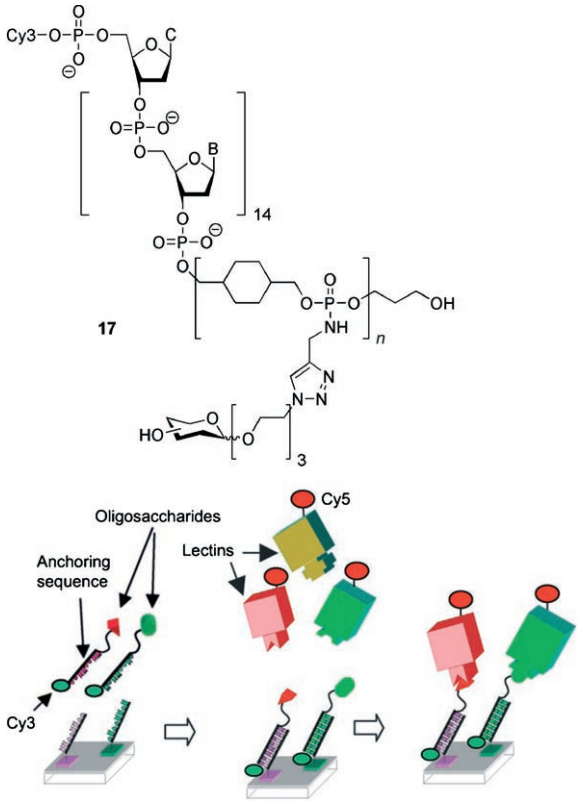
Scheme 4. The molecular toolbox for the synthesis of cyclic, branched cyclic, and bicyclic DNA introduced by Morvan and co-workers. ● = Solid resin, DMT = dimethoxytrityl.

group is stable to standard phosphoramidite coupling procedures and can be used to generate an azide group after the solid-phase synthesis by treatment with sodium azide.[28] Phosphoramidite **13** can analogously be used to produce DNA strands with an azide label on the 5′-terminus. Universal supports **14** and **15** can be used to introduce one or two 3′-terminal alkynes. This repertoire of building blocks allows access to a wide array of structures, such as DNA cycles and branched DNA cycles, as it is possible to label each position of DNA with alkynes as well as with azides. Even bicyclic DNA strands can be built up by using the double alkyne resin **15**. Phosphoramidite **12**, bearing two DMT-protected alcohols, can be used in the first elongation step to give a branched structure. Termination of the DNA synthesis with the bromoalkyl phosphoramidite **13** allows synthesis of a structure with two alkynes and two azides (**16**), which can be cyclized using CuAAC to give the desired bicyclic DNA species.

5. Analytical Applications of Modified DNA

Analytical applications of ODNs are vastly extended by the use of CuAAC chemistry owing to the higher yield and selectivity of the labeling reaction compared with classical methods. For example, conjugation to very hydrophobic dye structures can be achieved,[29] and the synthesis of highly modified DNA strands is possible, opening up a wide array of potential applications.

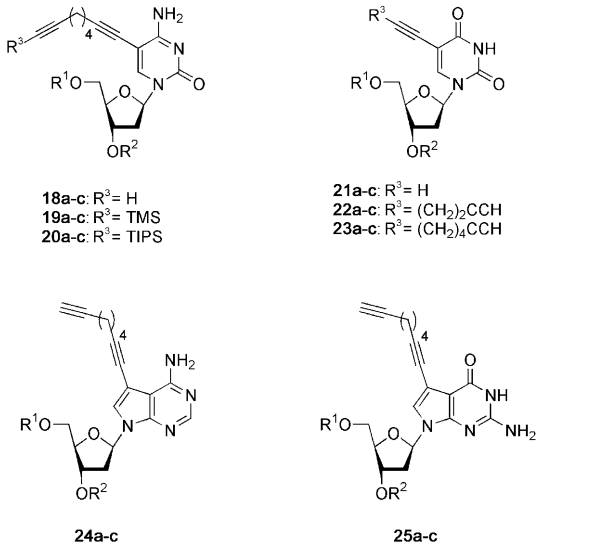
Morvan and co-workers synthesized oligonucleotide scaffolds (**17** in Scheme 5, top; $n = 1$ or 3) containing up to ten alkyne groups for the conjugation of galactose[30] and fucose



Scheme 5. DNA–carbohydrate conjugates (top, $n=1$ or 3) and their use in microarrays (bottom).[32]

to DNA strands.[31] In the latter case the binding of the fucose clusters to the fucose-specific bacterial lectin (PA-IIL) was up to 20 times stronger than for single fucose molecules. This technology was further developed for the preparation of DNA-based carbohydrate arrays (Scheme 5, bottom).[32] 3′-Amine oligonucleotides were covalently immobilized onto functionalized glass slides and then hybridized to DNA counter strands such as **17** bearing a sugar molecule attached by CuAAC at the 3′-terminus and a Cy3 fluorescence label at the 5′-terminus. The successful hybridization of the carbohydrate-modified DNA molecules to the DNA arrays could be monitored by resonant fluorescence energy transfer (FRET). The carbohydrate arrays constructed in this way were used to study the interaction with Cy5-labeled lectins. The interactions were investigated using FRET spectroscopy; the sensitivity of the method was found to be excellent, with detection limits for lectins between 2 and 20 nM.

Detection of DNA synthesis in vivo was recently also accomplished using CuAAC. To this end 5-ethynyl-2′-deoxyuridine **21a** (Scheme 6) was synthesized and injected into mice or added to a NIH 3T3 cell culture.[33] The structural similarity between the alkynyluridine **21a** and thymidine enabled the polymerase-based incorporation of the alkyne base into DNA during the S-phase of the cell cycle. Additional **21a** is incorporated into cell DNA in each cell cycle. After lysis of the cells the genomic DNA could be directly stained with an azide-modified fluorescent dye to quantify the rate of DNA synthesis in different tissues. This method was found to be more sensitive and user-friendly than the standard



Scheme 6. Nucleobase-modified phosphoramidites and triphosphates. **18a–25a**: $R^1=R^2=H$; **18b–25b**: $R^1=P_3O_9^{4-}$, $R^2=H$; **18c–25c**: $R^1=$DMT, $R^2=P(NiPr_2)[O(CH_2)_2CN]$. TIPS = triisopropylsilyl, TMS = trimethylsilyl.

bromodeoxyuridine (BrdU) assay, which requires a time-consuming immunological step involving specific anti-BrdU antibodies.

A very simple and sensitive DNA detection method based on the silver halide photographic process was presented by Carell and co-workers.[34] Photographic AgBr emulsions are generally sensitive at wavelengths of incident light up to 520 nm. Organic dyes are typically added to black and white photo paper to sensitize the photographic films over the whole visible range. The signal enhancement of the photographic development procedure with amplification factors of up to 10^{11} results in sensitivities similar to the polymerase chain reaction (PCR). In this study, a photographic film devoid of any sensitizing dyes (i.e. insensitive to light $\lambda >$ 520 nm) was treated with DNA incorporating either **18c** or **23c** (Scheme 6). The photographic red-light sensitizer (pinacyanol dye azide) was attached to these alkyne-containing nucleobases by CuAAC. After a short irradiation of the film with red light, only the parts of the film which contained the spotted-on DNA displayed a black spot after development, thus indicating that the dye present at the DNA selectively sensitized the AgBr crystals in its vicinity. The whole method allowed the visual detection of DNA with a detection limit of only 300 amol.

The extremely high sensitivity of the new photographic method was used to directly detect the DNA of pest-causing bacteria. To furnish a simple experimental setup for the detection system, a molecular beacon (MB)-based approach was devised (Figure 2). To this end, a commercially available molecular beacon with the Cy3/BHQ2 (black hole quencher) FRET couple was used, which featured a base sequence characteristic for *Yersinia pestis* in the loop region. In the closed state of the hairpin, the fluorescence of the Cy3 dye is quenched by BHQ2, which is in close proximity, and therefore cannot sensitize the photographic film. If the characteristic complementary sequence is present in the analysis mixture, it

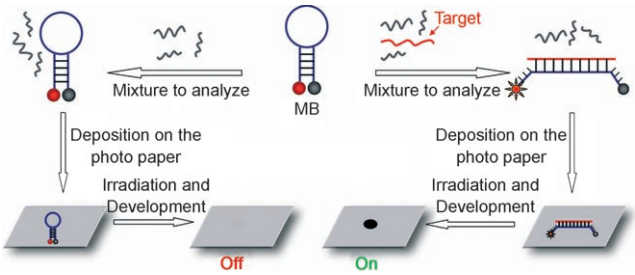


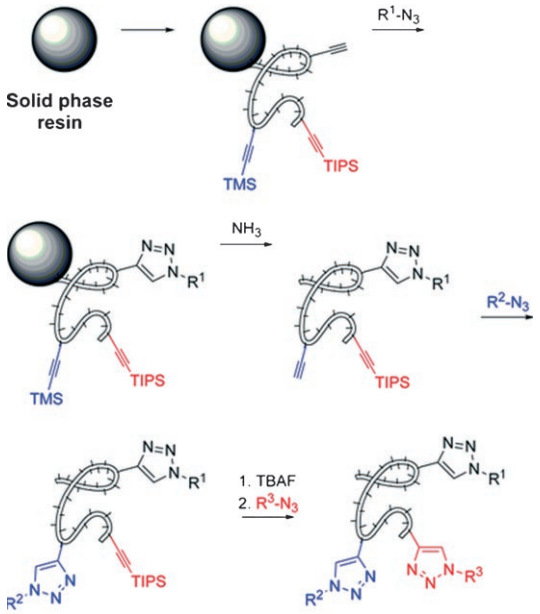
Figure 2. Working principle of the molecular-beacon-based DNA photography. Only the target molecule is detected.[34]

will hybridize to the loop region and open the hairpin, which immediately reactivates Cy3 fluorescence and sensitizing properties. If this solution is now spotted onto the sensitizer-free photographic film, the fluorescing Cy3-labeled DNA allows sensitization of the AgBr crystals after brief irradiation, which enables the visual detection of the analyte with a detection limit of 600 fmol. The control experiment without the complementary sequence yielded a blank film.

6. Multiple, Sequential Labeling of DNA

The attachment of alkynyl side chains to the 5-position of pyrimidines (**18**–**23** in Scheme 6) or to the 7-position of 7-deazapurines (**24** and **25** in Scheme 6) offers a variety of possibilities for the versatile labeling of DNA. These positions have the advantage that the corresponding alkyne-modified bases are generally well accepted by polymerases for enzymatic incorporation into PCR and primer extension products (Section 7).[35] Functional molecules attached to these positions of the nucleobases will protrude into the major groove of the B-duplex, which causes only small distortions of the duplex structures required for efficient hybridization with the counter strand. Indeed, such modified DNA strands exhibit only a small increase in melting temperature compared to natural DNA, showing the small effect of modifications at C5 of pyrimidines and C7 of 7-deazapurines on the duplex.[36,37] The choice of alkyne group influences the efficiency of subsequent modification. If a high functionalization density of single- or double-stranded DNA is required, the octadiynyl spacer protruding from these positions into the major group proved to be superior to the short ethynyl linker in **21**. The octadiynyl units position the triple bond somewhat further from the DNA strand, thus allowing for better steric access. Single-stranded ODNs with up to six consecutive octadiynyl uridine moieties **23c** were synthesized, and with this alkyne spacer quantitative yields in the CuAAC reaction were always detected with a variety of azides (fluorescein, coumarin, and galactose).[38]

The possibility to prepare a set of different alkyne-modified bases was then shown to be the basis for an efficient modification of DNA with different labels. Such multiply modified DNA strands find wide use, for example as sophisticated FRET probes or in molecular diagnostics applications. To this end, DNA strands containing TMS- (**19c**) and TIPS-protected (**20c**) alkyne 5-alkyne-2′-deoxycytidine analogues were prepared.[39] These two building blocks, together with the alkyne-modified uridine analogue **23c**, were incorporated into DNA strands. Three different CuAAC reactions were performed consecutively, first at the free alkyne of the uridine analogue, at the second alkyne residue after TMS deprotection, and finally at the third alkyne residue after TIPS deprotection (Scheme 7). This approach allowed the introduction of three different labels with unprecedented yield and efficiency. The CuAAC modification could even be achieved directly at the resin, allowing the derivatization of oligonucleotides using a DNA synthesizer. In many cases, a simple ethanol precipitation was sufficient to purify the labeled oligonucleotides with yields between 60 and 90% observed for the three-step click-deprotect-click reaction sequence in solution.



Scheme 7. Sequential modification of DNA.

7. Enzymatic Incorporation of Alkynes into DNA and DNA Metallization

The alkyne-modified triphosphates **18b**–**25b** can be efficiently incorporated into PCR products enzymatically. In a screening of different polymerases, *Pwo*, *Deep Vent exo*$^-$, and *KOD XL* polymerases were found to be particularly proficient at incorporating these unnatural nucleotide triphosphates.[36] Indeed, even the preparation of 2000-mer PCR products with all cytosine bases replaced by the alkyne-modified cytosine **18b** was reported. With a galactose azide, these highly modified DNA amplicons were subsequently converted into sugar-coated DNA strands possessing 887 sugar molecules attached to the DNA. Again, the longer and more flexible bisalkyne linkers present in **18**, **23**, **24**, and **25** were found to lead to higher CuAAC yields than the ethynyl-modified base **21**. A substitution of all four canonical bases by alkyne bases in a PCR reaction proved unfeasible, showing that the polymerases are not capable of placing an alkyne

nucleotide opposite to another alkyne nucleotide. In simple primer extension experiments, however, replacement of all four canonical bases was possible.[40] Most important, however, was the observation that the as-modified amplicons can function as templates for normal PCR reactions. Sequencing of these amplicons revealed no mutations, showing that the alkyne bases can be used to label PCR products, which can then be used for further cloning and PCR experiments.

An interesting application of the sugar-coated DNA is the fabrication of metallized DNA strands. (Figure 3). Carell and co-workers prepared 2000-mer DNA strands with all natural dT bases replaced by an alkyne-modified uridine **21b** or **22b**.[35] This DNA was subsequently modified with galactose azide using the CuAAC reaction. Treatment of the as-prepared DNA with Tollens solution allowed selective deposition of atomic silver on the DNA; after a developing reaction, this atomic silver can be developed to nanoparticles as in black-and-white photography. In a second step, the nanoparticles were exposed to a gold enhancer solution (AuSCN, hydroquinone)[41] to achieve the deposition of a thin coating of gold metal around the DNA strand. The resulting gold–DNA nanowires were shown to have an extremely uniform diameter that can be limited to less than or equal to 10 nm by controlling the time of development. Even the time-dependent growth of the gold mantle around the DNA could be studied by AFM on silica and mica (Figure 4). Using the CuAAC reaction, it was recently also possible to deposit size-uniform gold nanoparticles to the backbone of alkyne-containing PCR products. For this purpose the CuAAC reaction was performed with a gold nanoparticle that was bound to an azide-modified glutathione ligand. Again, the gold nanoparticles exhibited a very uniform distribution along the DNA scaffold.[42]

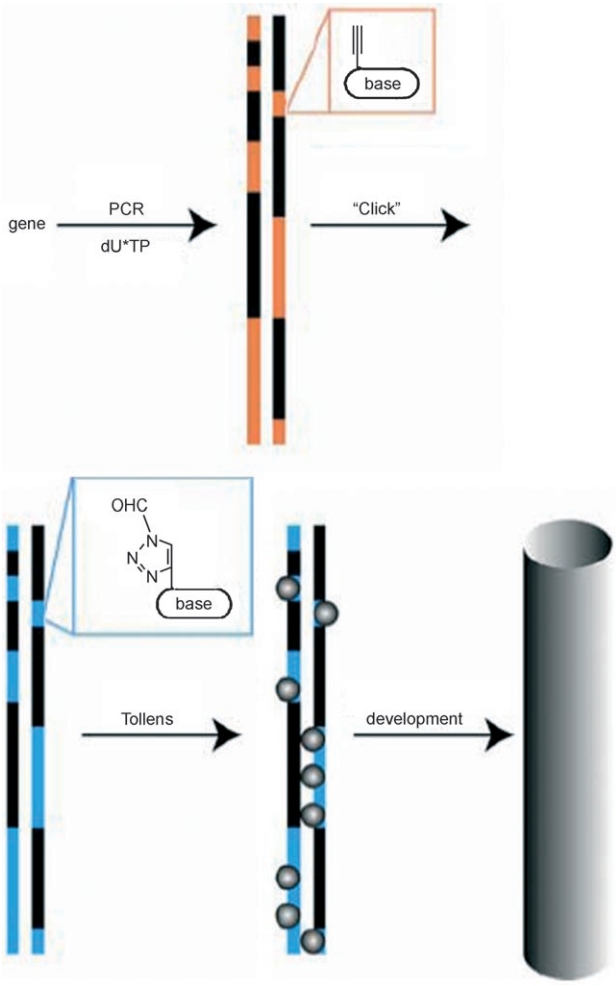


Figure 3. Schematic representation of metal deposition on PCR products.

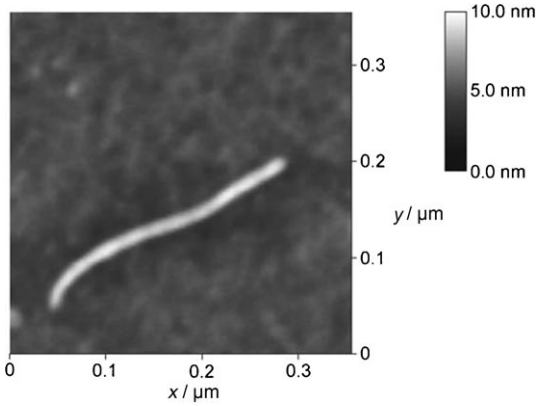
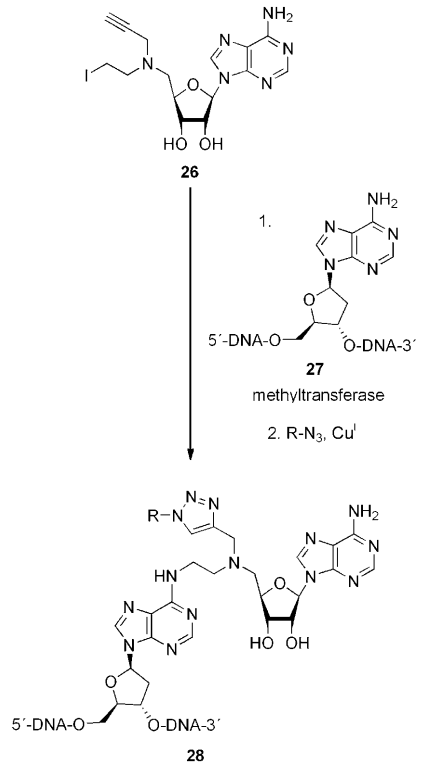


Figure 4. AFM images of bimetallic silver–gold nanowires deposited on sugar-decorated DNA.[41]

Most recently, Weller and Rajski[43] developed an ingenious method to label large DNA fragments site-selectively using the CuAAC reaction based on seminal discoveries of Weinhold an co-workers.[44] This group employed methyltransferases (MTases), which catalyze the transfer of an activated methyl group from the ubiquitous cofactor S-adenosyl-L-methinone (AdoMet or SAM) to adenine or cytosine residues on DNA within specific DNA sequences between two and eight base pairs long.[45] Similar to Weinhold and co-workers,[44] Weller and Rajski prepared the novel cofactor **26**, which is accepted by the methyltransferase M.TaqI instead of natural SAM (Scheme 8). With the novel cofactor, however, not a methyl group but a propargyl-modified adenine moiety was transferred to the N6 nitrogen of adenine in the palindromic sequence 5′-AGCT-3′. The resulting DNA is thus selectively labeled with an alkynyl adenine moiety at each AGCT site. The alkyne group was then further functionalized with azides using the copper(I)-catalyzed Huisgen reaction. This method is not restricted by the length of the DNA strand and could thereby lead to the assembly of alkyne-labeled genomic DNA with a low density of functionalization.

8. Summary and Outlook

The copper-catalyzed azide–alkyne cycloaddition (CuAAC) has changed the way in which modified oligonucleotides can be prepared. It is possible to attach all kinds of labels to small oligonucleotides using alkyne-modified phos-



Scheme 8. MTase-mediated decoration of DNA with alkyne groups.

phoramidites. One, two, or even three different labels can be attached either in solution or on a solid support. With the help of alkyne-modified triphosphates or using the methyltransferase technology, large PCR fragments of genomic DNA containing one or multiple alkyne sites can be readily prepared, ready for modification by CuAAC. It is astonishing that many alkyne-modified bases are readily accepted by polymerases, thus allowing the full replacement of the canonical bases in an amplicon by alkyne-modified bases. The alkyne-bearing DNA can be modified in extremely high yields using the CuAAC reaction. Many of the click-modified oligonucleotides are easily purified using simple ethanol precipitation procedures, thus circumventing time-consuming and expensive HPLC purification protocols. The alkyne-modified DNA strands can be used as tools for modern molecular diagnostic applications, and they can act as building blocks for the construction of functional DNA-based nanomaterials ready for modification with all types of functional molecular entities. For DNA chemists working in the fields of diagnostics, medicine, and nanotechnology, the CuAAC reaction on DNA has vastly broadened the synthesis capabilities, paving the way for the preparation of totally new DNA-based molecules and nanosystems.

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[1] R. Huisgen, *1,3-Dipolar Cycloaddition Chemistry*, *Vol. 1*, Wiley, New York, **1984**, pp. 1–176.
[2] J.-F. Lutz, *Angew. Chem.* **2007**, *119*, 1036–1043; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018–1025; V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51–68.
[3] C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064; 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.
[4] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128–1137.
[5] V. Aucagne, D. A. Leigh, *Org. Lett.* **2006**, *8*, 4505–4507; H. T. Ten Brink, J. T. Meijer, R. V. Geel, M. Damen, D. W. P. M. Löwik, J. C. M. van Hest, *J. Pept. Sci.* **2006**, *12*, 686–692.
[6] A. E. Speers, B. F. Cravett, *Chem. Biol.* **2004**, *11*, 535–546.
[7] T. Liebert, C. Hänsch, T. Heinze, *Macromol. Rapid Commun.* **2006**, *27*, 208–213.
[8] Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn, *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193.
[9] J. A. Link, D. A. Tirrell, *J. Am. Chem. Soc.* **2003**, *125*, 11164–11165.
[10] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109–1151; S. Thyagarajan, N. N. Murthy, A. A. N. Sarjeant, K. D. Karlin, S. E. Rokita, *J. Am. Chem. Soc.* **2006**, *128*, 7003–7008.
[11] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853–2855.
[12] L. M. Smith, S. Fung, M. W. Hunkapiller, T. J. Hunkapiller, L. E. Hood, *Nucleic Acids Res.* **1985**, *13*, 2399–2412.
[13] M. Kuwahara, Y. Takahata, A. Shoji, A. N. Ozaki, H. Ozaki, H. Sawai, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3735–3738; A. Roychowdhury, H. Illangkoon, C. L. Hendrickson, S. A. Benner, *Org. Lett.* **2004**, *6*, 489–492; M. Mehedi Masud, M. Kuwahara, H. Ozaki, H. Sawai, *Bioorg. Med. Chem.* **2004**, *12*, 1111–1120.
[14] I. Géci, V. V. Filichev, E. B. Pedersen, *Chem. Eur. J.* **2007**, *13*, 6379–6386.
[15] D. I. Rozkiewicz, J. Gierlich, G. A. Burley, K. Gutsmiedl, T. Carell, B. J. Ravoo, D. N. Reinhoudt, *ChemBioChem* **2007**, *8*, 1997–2002.
[16] A. M. Jawalekar, N. Meeuwenoord, J. G. O. Cremers, H. S. Overkleeft, G. A. van der Marel, F. P. J. T. Rutjes, F. L. v. Delft, *J. Org. Chem.* **2008**, *73*, 287–290.
[17] T. S. Seo, Z. Li, H. Ruparel, J. Ju, *J. Org. Chem.* **2003**, *68*, 609–612.
[18] Z. Li, T. S. Seo, J. Ju, *Tetrahedron Lett.* **2004**, *45*, 3143–3146.
[19] T. S. Seo, X. Bai, H. Ruparel, Z. Li, N. J. Turro, J. Ju, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5488–5493; T. S. Seo, X. Bai, D. H. Kim, Q. Meng, S. Shi, H. Ruparel, Z. Li, N. J. Turro, J. Ju, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5926–5931.
[20] N. K. Devaraj, G. P. Miller, W. Ebina, B. Kakaradov, J. P. Collman, E. T. Kool, C. E. D. Chidsey, *J. Am. Chem. Soc.* **2005**, *127*, 8600–8601.
[21] S. Mazzini, F. Garcia-Martin, M. Alvira, A. Avino, B. Manning, F. Albericio, R. Eritja, *Chem. Biodiversity* **2008**, *5*, 209–218; M. Alvira, R. Eritja, *Chem. Biodiversity* **2007**, *4*, 2798–2809; K. Gogoi, M. V. Mane, S. S. Kunte, V. A. Kumar, *Nucleic Acids Res.* **2007**, *35*, e139; M. Humenik, Y. Huang, Y. Wang, M. Sprinzl, *ChemBioChem* **2007**, *8*, 1103–1106.

[22] B. P. Duckworth, Y. Chen, J. W. Wollack, Y. Sham, J. D. Mueller, T. A. Taton, M. D. Distefano, *Angew. Chem.* **2007**, *119*, 8975 – 8978; *Angew. Chem. Int. Ed.* **2007**, *46*, 8819 – 8822.
[23] R. P. Goodman, I. A. T. Schaap, C. F. Tardin, C. M. Erben, R. M. Berry, C. F. Schmidt, A. J. Turberfield, *Science* **2005**, *310*, 1661 – 1665.
[24] M. Nakane, S. Ichikawa, A. Matsuda, *Nucleic Acids Symp. Ser.* **2005**, *49*, 189 – 190.
[25] R. Kumar, A. El-Sagheer, J. Tumpane, P. Lincoln, L. M. Wilhelmson, T. Brown, *J. Am. Chem. Soc.* **2007**, *129*, 6859 – 6864.
[26] M. Nakane, S. Ichikawa, A. Matsuda, *J. Org. Chem.* **2008**, *73*, 1842 – 1851.
[27] J. Lietard, A. Meyer, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2008**, *73*, 191 – 200; A. Meyer, C. Bouillon, S. Vidal, J.-J. Vasseur, F. Morvan, *Tetrahedron Lett.* **2006**, *47*, 8867 – 8871.
[28] J. Lietard, A. Meyer, J.-J. Vasseur, F. Morvan, *Tetrahedron Lett.* **2007**, *48*, 8795 – 8798.
[29] A. V. Ustinov, V. V. Dubnyakova, V. A. Korshun, *Tetrahedron* **2008**, *64*, 1467 – 1473; A. V. Ustinov, V. A. Korshun, *Russ. Chem. Bull.* **2006**, *55*, 1268 – 1274.
[30] C. Bouillon, A. Meyer, S. Vidal, A. Jochum, Y. Chevolot, J.-P. Cloarec, J.-P. Praly, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2006**, *71*, 4700 – 4701.
[31] F. Morvan, A. Meyer, A. Jochum, C. Sabin, Y. Chevolot, A. Imberty, J.-P. Praly, J.-J. Vasseur, E. Souteyrand, S. Vidal, *Bioconjugate Chem.* **2007**, *18*, 1637 – 1643.
[32] Y. Chevolot, C. Bouillon, S. Vidal, F. Morvan, A. Meyer, J.-P. Cloarec, A. Jochum, J.-P. Praly, J.-J. Vasseur, E. Souteyrand, *Angew. Chem.* **2007**, *119*, 2450 – 2454; *Angew. Chem. Int. Ed.* **2007**, *46*, 2398 – 2402.
[33] A. Salic, T. J. Mitchison, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2415 – 2420.
[34] D. M. Hammond, A. Manetto, J. Gierlich, V. A. Azov, P. M. E. Gramlich, G. A. Burley, M. Maul, T. Carell, *Angew. Chem.* **2007**, *119*, 4262 – 4265; *Angew. Chem. Int. Ed.* **2007**, *46*, 4184 – 4187.
[35] G. A. Burley, J. Gierlich, M. R. Mofid, S. T. H. Nir, Y. Eichen, T. Carell, *J. Am. Chem. Soc.* **2006**, *128*, 1398 – 1399.
[36] J. Gierlich, K. Gutsmiedl, P. M. E. Gramlich, A. Schmidt, G. Burley, T. Carell, *Chem. Eur. J.* **2007**, *13*, 9486 – 9494.
[37] F. Seela, V. R. Sirivolu, *Helv. Chim. Acta* **2007**, *90*, 535 – 552; F. Seela, V. R. Sirivolu, *Chem. Biodiversity* **2006**, *3*, 509 – 514; F. Seela, V. R. Sirivolu, P. Chittepu, *Bioconjugate Chem.* **2008**, *19*, 211 – 224; F. Seela, V. R. Sirivolu, *Nucleosides Nucleotides Nucleic acids* **2007**, *26*, 597 – 601; P. Kocalka, N. K. Andersen, F. Jensen, P. Nielsen, *ChemBioChem* **2007**, *8*, 2106 – 2116.
[38] J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639 – 3642.
[39] P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem.* **2008**, *120*, 3491 – 3493; *Angew. Chem. Int. Ed.* **2008**, *47*, 3442 – 3444.
[40] P. M. E. Gramlich, C. T. Wirges, J. Gierlich, T. Carell, *Org. Lett.* **2008**, *10*, 249 – 251.
[41] M. Fischler, U. Simon, H. Nir, Y. Eichen, G. A. Burley, J. Gierlich, P. M. E. Gramlich, T. Carell, *Small* **2007**, *3*, 1049 – 1055.
[42] M. Fischler, A. Sologubenko, J. Mayer, G. Clever, G. Burley, J. Gierlich, T. Carell, U. Simon, *Chem. Commun.* **2008**, 169 – 171.
[43] R. L. Weller, S. R. Rajski, *Org. Lett.* **2005**, *7*, 2141 – 2144; R. L. Weller, S. R. Rajski, *ChemBioChem* **2006**, *7*, 243 – 245.
[44] M. Pignot, C. Siethoff, M. Linscheid, E. Weinhold, *Angew. Chem.* **1998**, *110*, 3050 – 3053; *Angew. Chem. Int. Ed.* **1998**, *37*, 2888 – 2891.
[45] G. Pljevaljcic, F. Schmidt, A. J. Scheidig, R. Lurz, E. Weinhold, *ChemBioChem* **2007**, *8*, 1516 – 1519.

