Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Reactive modifications of DNA nucleobases for labelling, bioconjugations, and cross-linking

Ivana Ivancová[1,2], Denise-Liu' Leone[1,2] and Michal Hocek[1,2]

Modification of DNA with reactive groups and their post-synthetic transformations are useful for labelling, imaging, bioconjugations and cross-linking with other (bio)molecules. This review summarizes the recent progress in this field and covers transformations of oxo groups, cycloadditions, conjugate additions, alkylations, cross-couplings and other reactions. Examples of applications are given and the practicability and scope of the reactions are discussed.

Addresses
[1] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nam. 2, 16610 Prague 6, Czech Republic
[2] Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, CZ-12843 Prague 2, Czech Republic

Corresponding author: Hocek, Michal (hocek@uochb.cas.cz)

Current Opinion in Chemical Biology 2019, **52**:136–144

This review comes from a themed issue on **Synthetic biomolecules**

Edited by **Johan Winne** and **Annemieke Madder**

For a complete overview see the Issue and the Editorial

Available online 12th August 2019

https://doi.org/10.1016/j.cbpa.2019.07.007

Introduction

Bioconjugations and labelling of biomolecules are important for many applications in medicinal chemistry, molecular and chemical biology, imaging and diagnostics. The busy field of bioconjugations of nucleic acids has been thoroughly reviewed several times [1–4]. This review will summarize postsynthetic modifications of DNA through reactions of modified nucleobases. It will not cover 3′-end or 5′-end modifications of DNA [1–3] or modifications of RNA [5•], which have been reviewed very recently. The review will discuss the scope and practicability of the reactions for applications in labelling, bioconjugations and cross-linking of DNA with reporting groups or other biomolecules. It will focus on recent progress in the last three years, although some most important older reactions will be also mentioned and discussed for comparison. It is also complementary to our recent review [6] summarizing the use of base-modified DNA in studying of protein–DNA interactions.

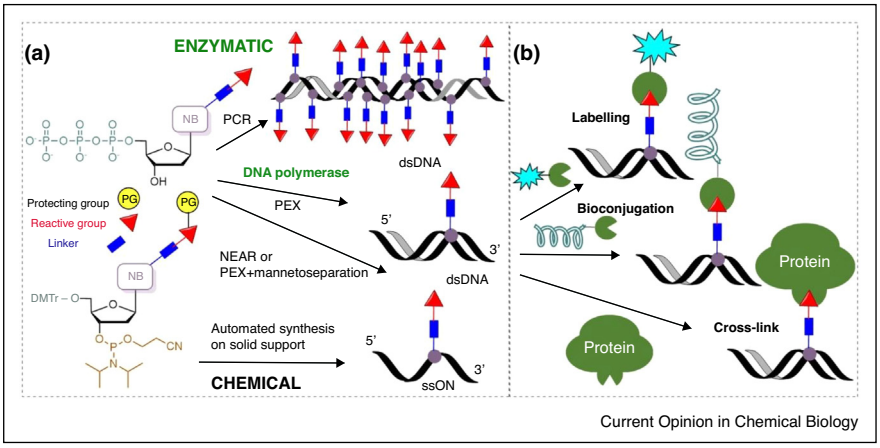
The starting modified oligonucleotides (ONs) and DNA can be synthesized either chemically or enzymatically (Scheme 1). The automated chemical synthesis is performed on solid support using modified nucleoside phosphoramidites [7]. Unfortunately, many reactive groups (electrophiles, nucleophiles etc.) are not compatible with the phosphoramidite protocol and/or deprotection with ammonia, and therefore often they must be masked or protected. On the other hand, the enzymatic synthesis using modified 2′-deoxyribonucleoside triphosphates (dNTPs) and DNA polymerases can use directly reactive-modified nucleotides for incorporation [8]. Different enzymatic methods, such as primer extension (PEX), PCR or nicking enzyme amplification reaction can be used for enzymatic synthesis of double-stranded or single-stranded DNA or ONs containing one, several or many modified nucleotides. These modified DNA or ONs can then undergo the postsynthetic modifications which are the topic of this review.

Postsynthetic modification of DNA by transformations of oxo groups

Reactions of oxo derivatives, in particular aldehydes, in ON and DNA have been extensively studied. In Nature, there exist at least two aldehyde-linked nucleobases. 5-Formylcytosine (5fC) is a rare epigenetic base which is an intermediate in active demethylation but it also can be an epigenetic signal on itself [9]. Related 5-formyluracil (5fU) occurs even less frequently as a product of oxidative damage of DNA but also its epigenetic role in some genomes has been suggested [10••]. For identification and mapping of the occurrence of these two rare natural nucleobases, number of specific reagents and reactions have been developed (Scheme 2).

Oxime or hydrazone formation is widely used in bioconjugations of aldehydes and ketones [11]. In DNA, oxime formation with fluorescent hydroxylamines was reported for fluorescent labelling of 5fC or 5fU (Scheme 2a), [12,13] as well as for labelling of these bases with biotin [14] or adamantine [15]. Hydrazone formation with nitrobenzofurazane-linked [16], naphthalimide-linked [17] or biotin-linked [14] hydrazines was used for staining or labelling of 5fU or 5fC in similar way (Scheme 2b). Although the oxime or hydrazone formation in water is not favored, some of these reactions were optimized to give almost quantitative yields. On the one hand, Schiff-base formation of 5fC with lysine of histone proteins has been

Scheme 1



Synthesis and postsynthetic modifications of functionalized ONs or DNA.

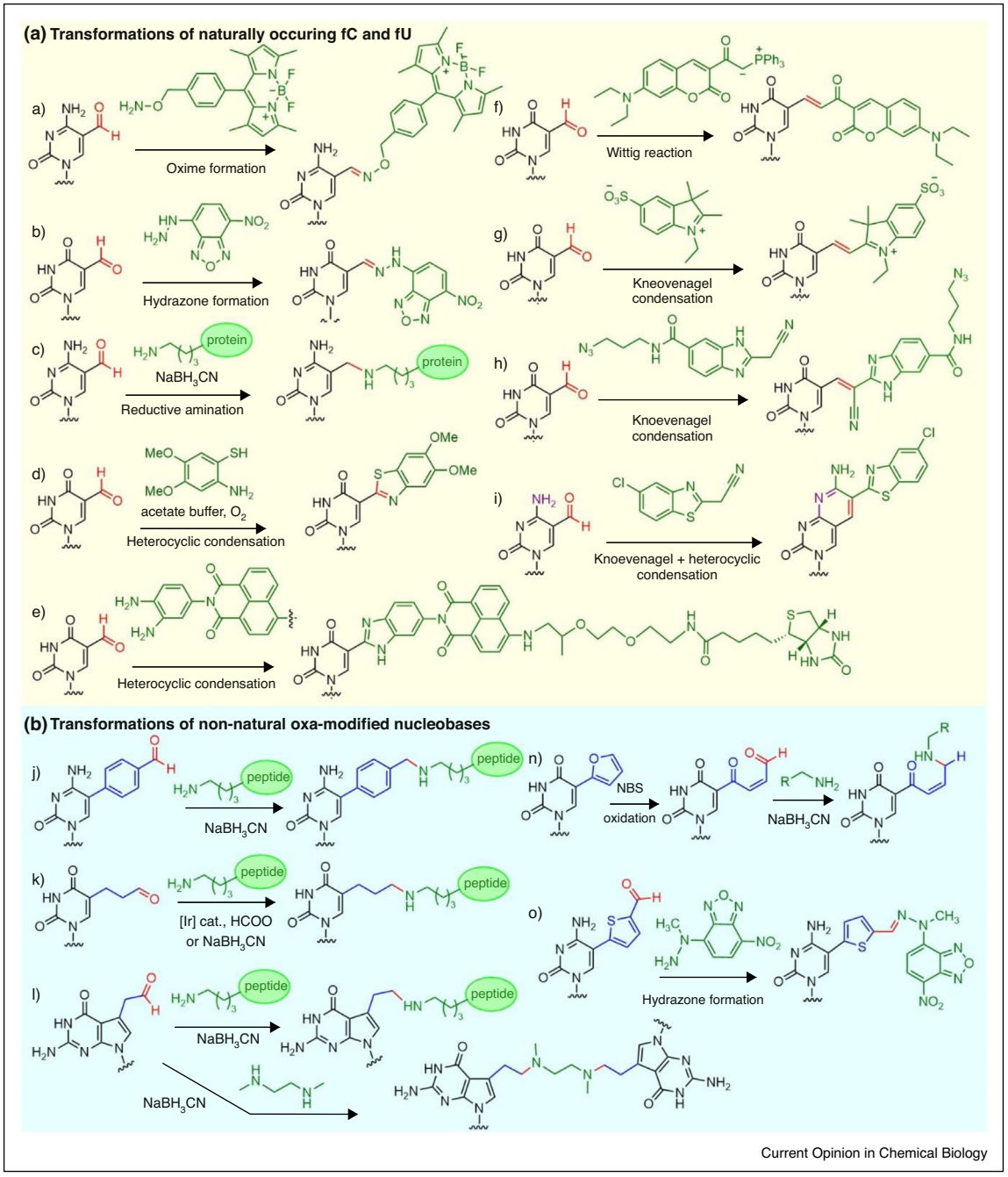
reported to form transient cross-links with equilibrium at max. 20% of the imine [18]. On the other hand, reductive amination of 5fC in the presence of $NaBH_3CN$ was used for permanent covalent cross-linking to histones [19••] and other proteins or peptides (Scheme 2c) [20]. Heterocyclizations of 5fU with substituted aminothiophenols or *o*-phenylenediamines formed fluorescent benzothiazole [21] or benzimidazole-linked [22] uracils are also useful for conjugation to biotin (Scheme 2d,e). Witting reaction with coumarin-based phosphine-ylide was used [23] for attachment of the fluorophore through vinylene group for cell imaging (Scheme 2f). Also Knoevenagel condensation of 5fU with active methylene reagents was used for fluorogenic formation of vinylene linked conjugates (Scheme 2g,h) [24,25]. In 5fC, the Knoevenagel condensation with benzothiazole-2-acetonitrile was followed by heterocyclization to the 4-amino group leading to fused pyridopyrimidine nucleobase (Scheme 2i) [26•], which is also fluorescent.

In addition to natural formylpyrimidines, number of other non-natural aldehyde-linked nucleobases have been prepared and used for bioconjugations. Formylphenylcytosine nucleotide was enzymatically incorporated to DNA and used for reductive amination (Scheme 2j) [27]. Aliphatic aldehyde-linked nucleobases were either incorporated to DNA [28] or generated in DNA through oxidative cleavage of vicinal diols and used for DNA metallization [29], conjugation to peptides or proteins [30] or inter-strand cross-linking [31] through reductive amination (Scheme 2k,l). Furan-linked uracil can be converted to highly reactive oxoaldehyde via reaction with NBS which was also used [32] for cross-linking with proteins through reductive amination (Scheme 2n). The formylthienylcytosine was also successfully used for staining through hydrazone formation (Scheme 2o) [33].

Postsynthetic modification of DNA by cycloaddition reactions

Cycloaddition reactions are one of the most useful and popular reactions for bioconjugations (Scheme 3). In particular, the Cu-catalyzed alkyne-azide cycloadditions (CuAAC), as the most important example of click reactions based on 1,3-dipolar cycloadditions, have been extensively used for bioconjugations and modifications of DNA and the field was reviewed several times [34,35]. For alkyne-azide click conjugations, the nucleobases in DNA can be modified either by terminal alkyne or by azido groups (Scheme 3a). The alkynes are compatible both with phosphoramidite synthesis and polymerase incorporations and a number of alkyne-nucleobase linked nucleoside phosphoramidites or dNTPs were used for synthesis of alkyne-linked DNA and follow-up CuAAC modifications in the major groove. Directly linked 5-ethynylpyrimidine or 7-ethynyl-7-deazapurine dNTPs are good substrates for DNA polymerases [36] and even some of the corresponding nucleosides are phosphorylated in the cells [37], which was used for staining of cellular DNA and metabolic labelling through CuAAC reactions with fluorophore-linked azides [38]. However, their CuAAC reactions of ethynylnucleobases in DNA are rather slow and do not give full conversions [39]. In contrast, octadiynyl-substituted pyrimidines [40], 7-deazapurines [41] or pyrazolopyrimidines [42] are much more reactive, but the dNTPs are weaker substrates for polymerases, in particular in PCR with long templates. Orthogonal silyl protection and release was used for selective triple CuAAC modifications of DNA [43]. Post-synthetic CuAAC click modification of ethynyl-linked DNA was recently successfully used for selection of aptamers [44•]. Recently, we have shown that propargyl-diethyleneglycol-linked thymidine triphosphate is an excellent substrate for polymerases and gives very fast and efficient CuAAC reactions [45].

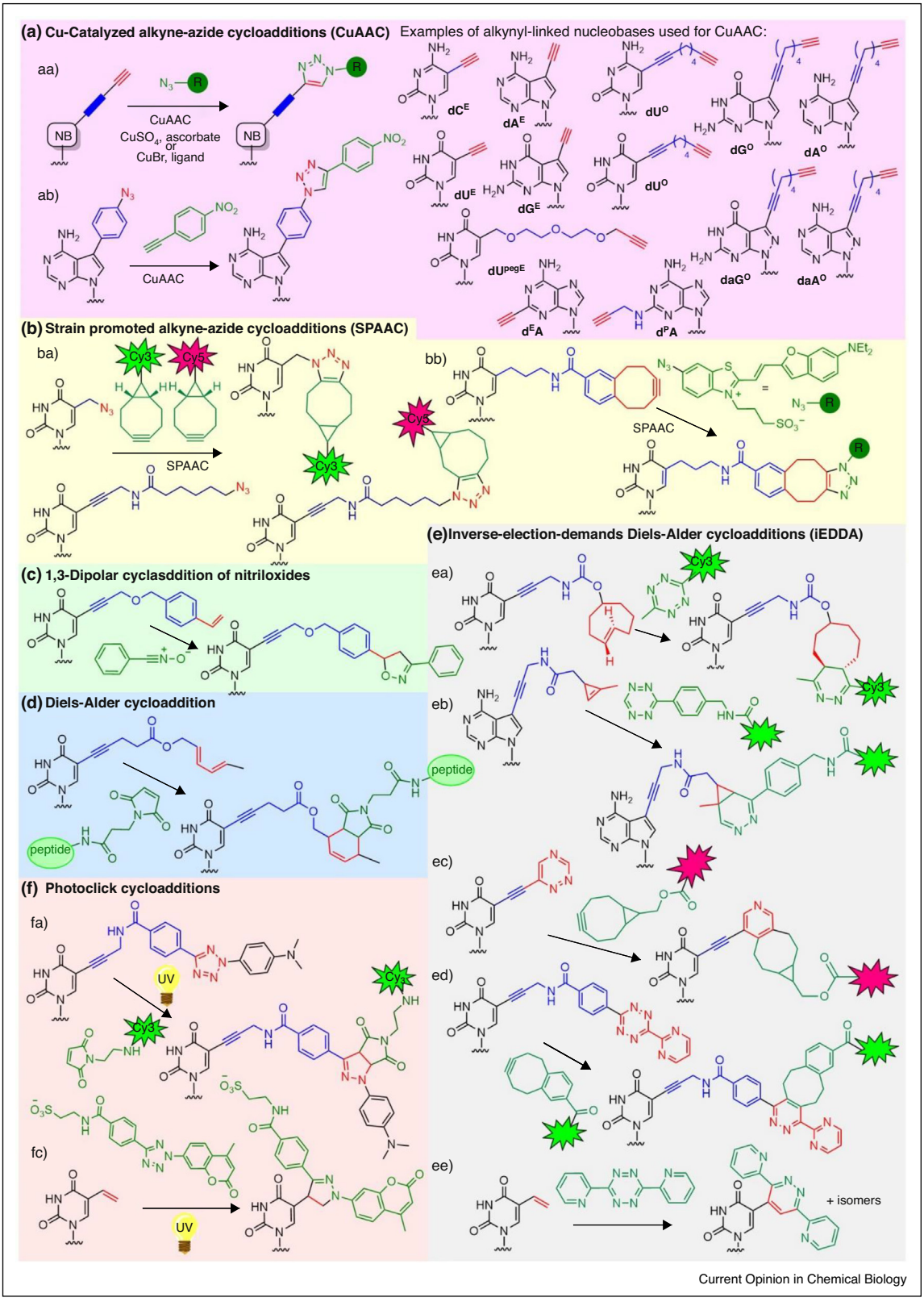
Scheme 2



Postsynthetic modifications of DNA through transformations of oxo groups.

Lately, also 2-ethynyl-[46] and 2-propargylamino-2′-deoxyadenosine [47] nucleotides were enzymatically incorporated to DNA and used for CuAAC reactions in the minor groove. Azido group is not compatible with phosphoramidite synthesis on solid support, but it can be still introduced to DNA by enzymatic incorporation of azido-modified nucleotides. Azidophenyl-linked dNTPs were prepared and used for polymerase synthesis of azido-modified DNA which was further transformed through CuAAC reaction with nitrophenylacetylene for redox labelling [48]. Cells treated with 5-azido-deoxyuridine or 5-azidomethyl-2′-deoxyuridine were able to phosphorylate and incorporate the azido-linked nucleotides to DNA which was then stained through CuAAC reaction with fluorescent alkynes for metabolic labelling [49].

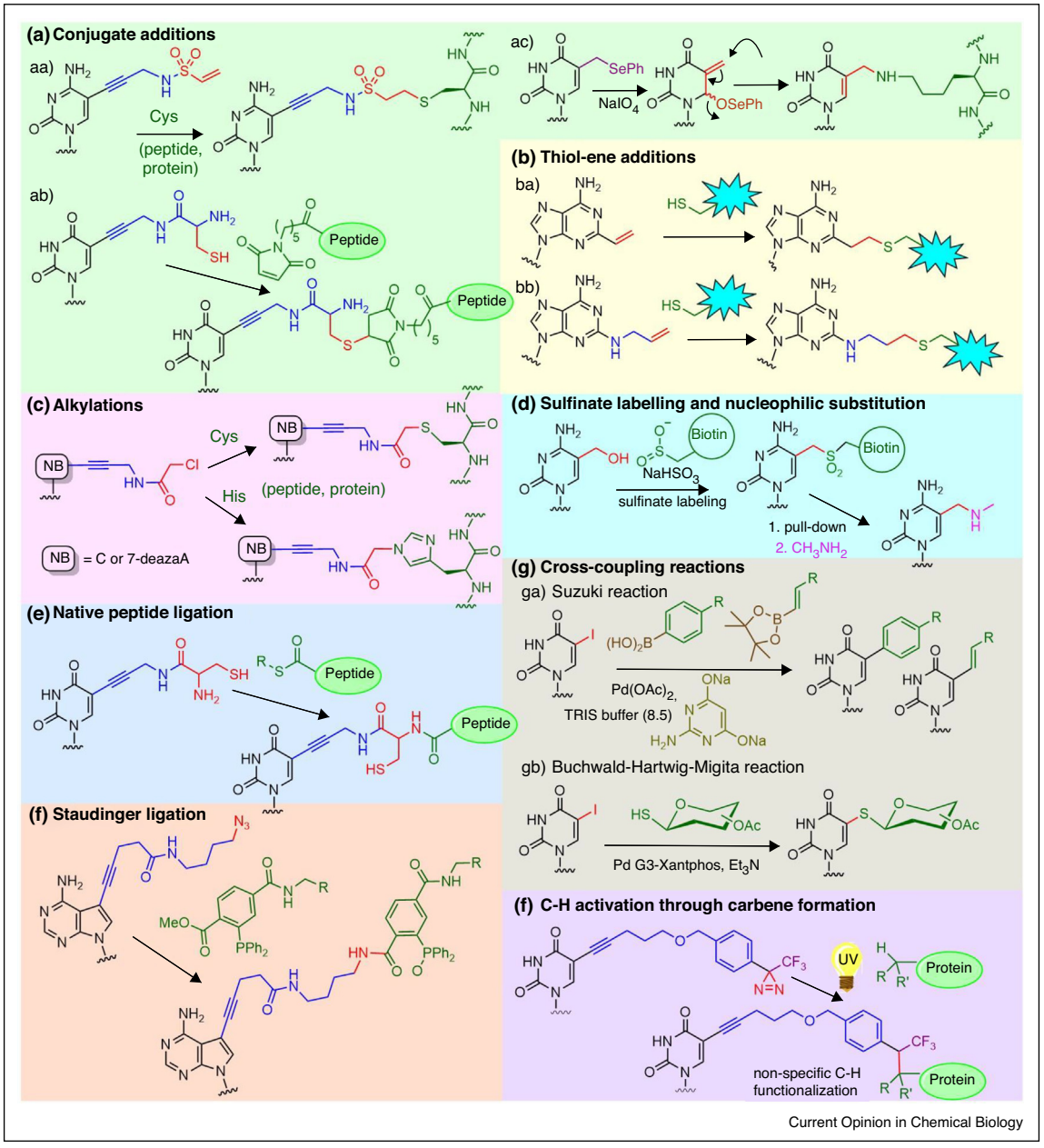
Scheme 3



Postsynthetic modifications of DNA through cycloaddition reactions.

Since the standard use of $CuSO_4$ with ascorbic acid causes DNA damage, the catalysis with CuBr in presence of chelating ligands is necessary for CuAAC modifications of DNA. However, even low concentration of copper cations can be toxic for living cells and therefore number of Cu-free click reactions have been developed in the last decade for prospective *in cellulo* and *in vivo* applications and their applications in DNA modifications have been reviewed in 2015 [50]. Copper-free alternative to CuAAC is the strain-promoted alkyne-azide cycloadditions (SPAAC) making use of strained alkynes (typically cyclooctyne derivatives) which are more reactive toward the 1,3-dipolar cycloadditions with azides without metal catalysis (Scheme 3b). Azido-linked dNTPs were synthesized and used for polymerase synthesis of DNA which underwent SPAAC reactions with cyclooctyne-linked fluorophores [51,52]. Azido group has also been introduced to DNA through another click reaction and was used for SPAAC with cyclooctyne-linked peptides [53]. Conversely, cyclooctyne nucleotides have been incorporated to DNA and used for SPAAC reactions with fluorescent azides [54] Also a 1,3-dipolar cycloaddition of alkenes with nitril-oxide was used for DNA modification (Scheme 3c) [55].

Scheme 4



Postsynthetic modifications of DNA through other types of reactions.

Another important type of click reactions is based on [4 + 2] Diels–Alder (DA) cycloadditions. Classical DA reaction of diene-linked nucleotide in DNA with maleimide-linked peptide was reported for synthesis of DNA-peptide conjugates (Scheme 3d) [56] Inverse-electron-demand Diels–Alder (iEDDA) reactions of alkenes with tetrazines or triazines have recently become one of the most important type of click reactions for bioconjugations due to its mild biocompatible conditions and bioorthogonality [57]. The iEDDA cycloaddition reaction is followed by spontaneous elimination of N_2 leading to dihydropyridazine or dihydropyridine (in case of reactions with alkenes) or to pyridazines or pyridines (in case of reactions with alkynes). DNA containing nucleobases bearing strained norbornene [58], *trans*-cyclooctene [52,59] or cyclopropane [60••] were synthesized and used for iEDDA click reactions with fluorescent tetrazines (Scheme 3e). Conversely, triazine-linked [60••] or tetrazine-linked [61] DNA was used for click reactions with fluorescent cyclooctynes. Even simple and easily accessible 5-vinyluracil [62] or 5-vinylcytosine [63] in DNA are sufficiently reactive with tetrazines, although mixtures of isomers are formed. Photochemically activated cycloadditions of tetrazoles with alkenes (photoclick reactions) have also successfully been used for DNA modification, either by incorporation of tetrazole-linked uracil and reaction with fluorescent maleimide [64] or by incorporation of vinyluracil and reaction with coumarin-linked tetrazole (Scheme 3f) [65].

Postsynthetic modification of DNA by other types of reactions

Aparts from the carbonyl and cycloaddition reactions discussed above, number of other reactions have been tested and used for postsynthetic modification of DNA (Scheme 4). Conjugate additions of thiols to vinylsulfonamide-linked DNA (prepared either by direct polymerase incorporation of modified nucleotide [66] or by CuAAC linking of the reactive group [67]) were used for cross-linking of DNA probes to p53 protein (Scheme 4aa). Conversely, thiol-linked DNA reacted [68] with maleimide-tethered peptides to form the DNA-peptide conjugates (Scheme 4ab). An interesting example was a Michael acceptor generated *in situ* by oxidation of phenylselenyl–thymine which cross-linked to lysine of histones (Scheme 4ac) [69] Photochemically induced thiol–ene additions of thiols to 2-vinyl-adenine [46] or 2-propargylamino-adenine [47] were used for attachment of fluorophores to minor groove of DNA (Scheme 4b). Polymerase incorporation of chloroacetamide-linked nucleotide resulted in reactive DNA probes which cross-linked [70] with Cys or His of interacting p53 protein making advantage of proximity effect (Scheme 4c). Naturally occurring 5-hydroxymethylcytosine underwent sulfinate labelling to attach biotin for pull-down and follow-up nucleophilic substitution with amine (Scheme 4d) [71]. Native chemical ligation of cysteine-tethered DNA with thioesters was used for conjugation of peptides (Scheme 4e) [72]. Staudinger reaction of an azido-linked nucleotide in DNA with *o*-diphenylphosphino-benzoate ester led to stable amide formation (Scheme 4f) [73].

Aqueous cross-coupling reactions are routinely used for modifications of nucleosides and nucleotides [8], but they can be also used for modification of DNA containing halogeno-substituted nucleobases (Scheme 4g), although their chemical incorporation to DNA by phosphoramidite protocol is non-trivial. 5-Iodouracil-containing ONs and DNA underwent the Suzuki-Miyaura cross-coupling with arylboronic acids or alenylborates in presence of Pd-catalysts and specific ligands to attach aryl or alkenyl groups (Scheme 4ga). [74,75] Also the Stille coupling with arylstannanes or alkenylstannanes was reported but this reaction needs large amounts of toxic triphenylarsine ligand [76]. The Buchwald–Hartwig–Migita reactions of 5-iodouracil in DNA with thiosugars were used for synthesis of DNA-glycoconjugates (Scheme 4gb) [77]. Diazirine group was attached to DNA either through incorporation of modified nucleotide [78] or through the Suzuki coupling [74] and its irradiation by UV light generated highly reactive carbene, which cross-linked to proteins via non-specific C–H bond activations (Scheme 4h).

Conclusions

The presented review summarized the recent progress in modification of DNA by reactive groups and their follow-up transformations for applications in labelling, imaging, bioconjugation and cross-linking. The reactions of oxo groups are most useful for detection and footprinting of naturally occurring epigenetic bases 5fC and 5fU, although their biological role has yet to be determined. Modification of DNA by non-natural aldehyde groups can be used for cross-linking to interacting proteins through reductive amination, though it requires additional reductant (e.g. $NaBH_3CN$). From cycloadditions, the CuAAC reactions of alkyne-linked DNA with azides have been the most frequently used transformations. The reactivity of the alkyne strongly depends on the linker and the use of Cu(I) is problematic in *in cellulo* and *in vivo* applications (due to toxicity of copper). Synthesis of azido-linked DNA is more difficult (azido group is not compatible with phosphoramidite protocol) but apart from CuAAC, the azido group can be used for Cu-free SPAAC. The SPAAC is more biocompatible but the synthesis of DNA modified by bulky strained alkynes is quite difficult, in particular the enzymatic synthesis because dNTPs bearing bulky groups are worse substrates for DNA polymerases (especially in PCR). iEDDA cycloadditions and photoclick reactions are also very useful and fully bioorthogonal reactions, but the synthesis of modified DNA may also be challenging. Modification of DNA with strong electrophiles, Michael acceptors or

alkylation agents is difficult but can be achieved by enzymatic incorporation of modified nucleotides. These electrophiles are highly reactive toward nucleophiles; hence the reactivity needs to be optimized to avoid non-specific reactions with abundant nucleophiles. Other transformations, such as thiol–ene additions, native ligations and Staudinger reaction have so far been used only for specific applications. Cross-coupling reactions have promising potential but yet they need to be further developed to become biocompatible. Non-specific cross-linking of diazirine-linked DNA with proteins has great potential in DNA-proteomics.

There is certainly a lot of space and need for improvement of the known and development of other reactions for modification of DNA, as well as for real *in vivo* application of some of these methods. New reactive modified nucleoside phosphoramidite building block need to be compatible with automated synthesis on solid support, whereas the dNTPs should be good substrates for DNA polymerases (which is problematic especially for bulkier functional groups) and the prospective new reactions should be bioorthogonal or very specific in the cellular environment containing mixture of many natural nucleophiles and other reactive species. Application of recently developed transporter [79••] with new modified dNTPs opens an attractive option of modification of genomic DNA in living cells. Also diverse reactivity toward natural amino acid side-chains is desirable for specific cross-linking DNA probes and for proteomics of DNA-interacting proteins. To this end, we have just developed a squaramate-modified nucleotides and DNA which cross-links with lysine-containing peptides or with histones without the need of an additional reagents (forming stable squaramide) [80]. Also some reverse reactions to release DNA from conjugates would be very useful for applications in gene delivery or regulation of gene expression [81•]. Research along these lines is on-going in our labs.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by the Academy of Sciences of the Czech Republic (Praemium Academiae to M. H.), the Czech Science Foundation (18-03305S to I. I., D.-L. L. and M. H.), and by the European Regional Development Fund; OP RDE (No. CZ.02.1.01/0.0/0.0/16_019/0000729 to M.H.).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Singh Y, Murat P, Defrancq E: **Recent developments in oligonucleotide conjugation**. *Chem Soc Rev* 2010, **39**:2054-2070 http://dx.doi.org/10.1039/b911431a.

2. Zatsepin TS, Stetsenko DA, Gait MJ, Oretskaya TS: **Use of carbonyl group addition-elimination reactions for synthesis of nucleic acid conjugates**. *Bioconjug Chem* 2005, **16**:471-489 http://dx.doi.org/10.1021/bc049712v.

3. Lönnberg H: **Solid-phase synthesis of oligonucleotide conjugates useful for delivery and targeting of potential nucleic acid therapeutics**. *Bioconjug Chem* 2009, **20**:1065-1094 http://dx.doi.org/10.1021/bc800406a.

4. Niemeyer CM: **Semisynthetic DNA-protein conjugates for biosensing and nanofabrication**. *Angew Chem Int Ed* 2010, **49**:1200-1216 http://dx.doi.org/10.1002/anie.200904930.

5. • Flamme M, McKenzie LK, Sarac I, Hollenstein M: **Chemical method for the modification of RNA**. *Methods* 2019, **161**:64-82 http://dx.doi.org/10.1016/j.ymeth.2019.03.018.
Important comprehensive review on chemical modifications of RNA.

6. Hocek M: **Enzymatic synthesis of base-functionalized nucleic acids for sensing, cross-linking, and modulation of protein-DNA binding and transcription**. *Acc Chem Res* 2019, **52**:1730-1737 http://dx.doi.org/10.1021/acs.accounts.9b00195.

7. Beaucage SL, Iyer RP: **Advances in the synthesis of oligonucleotides by the phosphoramidite approach**. *Tetrahedron* 1992, **48**:2223-2311 http://dx.doi.org/10.1016/S0040-4020(01)88752-4.

8. Hocek M: **Synthesis of base-modified 2′-deoxyribonucleoside triphosphates and their use in enzymatic synthesis of modified DNA for applications in bioanalysis and chemical biology**. *J Org Chem* 2014, **79**:9914-9921 http://dx.doi.org/10.1021/jo5020799.

9. Carell T, Kurz MQ, Müller M, Rossa M, Spada F: **Non-canonical bases in the genome: the regulatory information layer in DNA**. *Angew Chem Int Ed* 2018, **57**:4296-4312 http://dx.doi.org/10.1002/anie.201708228.

10. •• Raiber E-A, Hardisty R, van Delft P, Balasubramanian S: **Mapping and elucidating the function of modified bases in DNA**. *Nat Rev Chem* 2017, **1** http://dx.doi.org/10.1038/s41570-017-0069 0069.
Important comprehensive review on mapping and detection of epigenetic modifications in DNA.

11. Kölmel DK, Kool ET: **Oximes and hydrazones in bioconjugation: mechanism and catalysis**. *Chem Rev* 2017, **117**:10358-10376 http://dx.doi.org/10.1021/acs.chemrev.7b00090.

12. Guo P, Yan S, Hu J, Xing X, Wang C, Xu X, Qiu X, Ma W, Lu C, Weng X, Zhou X: **Selective detection of 5-formyl-2′-deoxycytidine in DNA using a fluorogenic hydroxylamine reagent**. *Org Lett* 2013, **15**:3266-3269 http://dx.doi.org/10.1021/ol401290d.

13. Liu C, Luo X, Chen Y, Fan W, Yang W, Wang Y, Zhang X, Zou G, Zhou X: **Selective labeling aldehydes in DNA**. *Anal Chem* 2018, **90**:14616-14621 http://dx.doi.org/10.1021/acs.analchem.8b04822.

14. Hardisty RE, Kawasaki F, Sahakyan AB, Balasubramanian S: **Selective chemical labeling of natural T modifications in DNA**. *J Am Chem Soc* 2015, **137**:9270-9272 http://dx.doi.org/10.1021/jacs.5b03730.

15. Wang S-R, Song Y-Y, Wei L, Liu C-X, Fu B-S, Wang J-Q, Yang X-R, Liu Y-N, Liu S-M, Tian T, Zhou X: **Cucurbit[7]uril-driven host–guest chemistry for reversible intervention of 5-formylcytosine-targeted biochemical reactions**. *J Am Chem Soc* 2017, **139**:16903-16912 http://dx.doi.org/10.1021/jacs.7b09635.

16. Liu C, Chen Y, Wang Y, Wu F, Zhang X, Yang W, Wang J, Chen Y, He Z, Zou G *et al.*: **A highly efficient fluorescence-based switch-on detection method of 5-formyluracil in DNA**. *Nano Res* 2017, **10**:2449-2458 http://dx.doi.org/10.1007/s12274-017-1445-2.

17. Wang Y, Liu C, Yang W, Zou G, Zhang X, Wu F, Yu S, Luo X, Zhou X: **Naphthalimide derivatives as multifunctional molecules for detecting 5-formylpyrimidine by both PAGE analysis and dot-blot assays**. *Chem Commun* 2018, **54**:1497-1500 http://dx.doi.org/10.1039/C7CC08715B.

18. Li F, Zhang Y, Bai J, Greenberg MM, Xi Z, Zhou C: **5-Formylcytosine yields DNA-protein cross-links in nucleosome**

core particles. *J Am Chem Soc* 2017, **139**:10617-10620 http://dx.doi.org/10.1021/jacs.7b05495.

19. •• Raiber E-A, Portella G, Martinez Cuesta S, Hardisty R, Murat P, Li Z, Iurlaro M, Dean W, Spindel J, Beraldi D *et al.*: **5-Formylcytosine organizes nucleosomes and forms Schiff base interactions with histones in mouse embryonic stem cells**. *Nat Chem* 2018, **10**:1258-1266 http://dx.doi.org/10.1038/s41557-018-0149-x.
This paper demonstrated for the first time that 5fC can reorganize histones through Shiff-base formation.

20. Ji S, Fu I, Naldiga S, Shao H, Basu AK, Broyde S, Tretyakova NY: **5-Formylcytosine mediated DNA-protein cross-links block DNA replication and induce mutations in human cells**. *Nucleic Acids Res* 2018, **46**:6455-6469 http://dx.doi.org/10.1093/nar/gky444.

21. Hirose W, Sato K, Matsuda A: **Selective detection of 5-formyl-2′-deoxyuridine, an oxidative lesion of thymidine, in DNA by a fluorogenic reagent**. *Angew Chem Int Ed* 2010, **49**:8392-8394 http://dx.doi.org/10.1002/anie.201004087.

22. Liu C, Wang Y, Zhang X, Wu F, Yang W, Zou G, Yao Q, Wang J, Chen Y, Wang S *et al.*: **Enrichment and fluorogenic labelling of 5-formyluracil in DNA**. *Chem Sci* 2017, **8**:4505-4510 http://dx.doi.org/10.1039/c7sc00637c.

23. Zhou Q, Li K, Liu Y-H, Li L-L, Yu K-K, Zhang H, Yu X-Q: **Fluorescent wittig reagent as a novel ratiometric probe for the quantification of 5-formyluracil and its application in cell imaging**. *Chem Commun* 2018, **54**:13722-13725 http://dx.doi.org/10.1039/c8cc07541g.

24. Samanta B, Seikowski J, Höbartner C: **Fluorogenic labeling of 5-formylpyrimidine nucleotides in DNA and RNA**. *Angew Chem Int Ed* 2016, **55**:1912-1916 http://dx.doi.org/10.1002/anie.201508893.

25. Wang Y, Liu C, Wu F, Zhang X, Liu S, Chen Z, Weiwu Z, Yang W, Zhang X, Zhou Y *et al.*: **Highly selective 5-formyluracil labeling and genome-wide mapping using (2-Benzimidazolyl) acetonitrile probe**. *iScience* 2018, **9**:423-432 http://dx.doi.org/10.1016/j.isci.2018.10.024.

26. • Liu C, Wang Y, Yang W, Wu F, Zeng W, Chen Z, Huang J, Zou G, Zhang X, Wang S *et al.*: **Fluorogenic labeling and single-base resolution analysis of 5-formylcytosine in DNA**. *Chem Sci* 2017, **8**:7443-7447 http://dx.doi.org/10.1039/c7sc03685j.
In this paper, fluorogenic labelling was used for detection of epigenetic base 5fC in genomic DNA.

27. Raindlová V, Pohl R, Hocek M: **Synthesis of aldehyde-linked nucleotides and DNA and their bioconjugations with lysine and peptides through reductive amination**. *Chem Eur J* 2012, **18**:4080-4087 http://dx.doi.org/10.1002/chem.201103270.

28. Krömer M, Bártová K, Raindlová V, Hocek M: **Synthesis of dihydroxyalkynyl and dihydroxyalkyl nucleotides as building blocks or precursors for introduction of diol or aldehyde groups to DNA for bioconjugations**. *Chem Eur J* 2018, **24**:11890-11894 http://dx.doi.org/10.1002/chem.201802282.

29. Wirges CT, Timper J, Fischler M, Sologubenko AS, Mayer J, Simon U, Carell T: **Controlled nucleation of DNA metallization**. *Angew Chem Int Ed* 2009, **48**:219-223 http://dx.doi.org/10.1002/anie.200803123.

30. Wickramaratne S, Mukherjee S, Villalta PW, Schärer OD, Tretyakova NY: **Synthesis of sequence-specific DNA-protein conjugates via a reductive amination strategy**. *Bioconjug Chem* 2013, **24**:1496-1506 http://dx.doi.org/10.1021/bc400018u.

31. Mukherjee S, Guainazzi A, Schärer OD: **Synthesis of structurally diverse major groove DNA interstrand crosslinks using three different aldehyde precursors**. *Nucleic Acids Res* 2014, **42**:7429-7435 http://dx.doi.org/10.1093/nar/gku328.

32. Carrette LLG, Gyssels E, Laet ND, Madder A: **Furan oxidation based cross-linking: a new approach for the study and targeting of nucleic acid and protein interactions**. *Chem Commun* 2016, **52**:1539-1554 http://dx.doi.org/10.1039/c5cc08766.

33. Raindlová V, Pohl R, Sanda M, Hocek M: **Direct polymerase synthesis of reactive aldehyde-functionalized DNA and its conjugation and staining with hydrazines**. *Angew Chem Int Ed* 2010, **49**:1064-1066 http://dx.doi.org/10.1002/anie.200905556.

34. Gramlich PME, Wirges CT, Manetto A, Carell T: **Postsynthetic DNA modification through the copper-catalyzed azide-alkyne cycloaddition reaction**. *Angew Chem Int Ed* 2008, **47**:8350-8358 http://dx.doi.org/10.1002/anie.200802077.

35. Amblard F, Cho JH, Schinazi RF: **Cu(I)-catalyzed Huisgen azide-alkyne 1,3-dipolar cycloaddition reaction in nucleoside, nucleotide, and oligonucleotide chemistry**. *Chem Rev* 2009, **109**:4207-4220 http://dx.doi.org/10.1021/cr9001462.

36. Cahová H, Panattoni A, Kielkowski P, Fanfrlík J, Hocek M: **5-Substituted pyrimidine and 7-substituted 7-deazapurine dNTPs as substrates for DNA polymerases in competitive primer extension in the presence of natural dNTPs**. *ACS Chem Biol* 2016, **11**:3165-3171 http://dx.doi.org/10.1002/anie.201609007.

37. Neef AB, Pernot L, Schreier VN, Scapozza L, Luedtke NW: **A bioorthogonal chemical reporter of viral Infection**. *Angew Chem Int Ed* 2015, **54**:7911-7914 http://dx.doi.org/10.1002/anie.201500250.

38. Salic A, Mitchison TJ: **A chemical method for fast and sensitive detection of DNA synthesis in vivo**. *Proc Natl Acad Sci U S A* 2008, **19**:2415-2420 http://dx.doi.org/10.1073/pnas.0712168105.

39. Slavíčková M, Janoušková M, Šimonová A, Cahová H, Kamková M, Šanderová H, Krásný L, Hocek M: **Turning off transcription with bacterial RNA polymerase through CuAAC click reactions of DNA containing 5-ethynyluracil**. *Chem Eur J* 2018, **24**:8311-8314 http://dx.doi.org/10.1002/chem.201801757.

40. Burley GA, Gierlich J, Mofid MR, Nir H, Tal S, Eichen Y, Carell T: **Directed DNA metallization**. *J Am Chem Soc* 2006, **128**:1398-1399 http://dx.doi.org/10.1021/ja055517v.

41. Gramlich PME, Wirges CT, Gierlich J, Carell T: **Synthesis of modified DNA by PCR with alkyne-bearing purines followed by a click reaction**. *Org Lett* 2008, **10**:249-251 http://dx.doi.org/10.1021/ol7026015.

42. Pujari SS, Ingale SA, Seela F: **High-density functionalization and cross-linking of DNA: "click" and "bis-click" cycloadditions performed on alkynylated oligonucleotides with fluorogenic anthracene azides**. *Bioconjug Chem* 2014, **25**:1855-1870 http://dx.doi.org/10.1021/bc5003532.

43. Gramlich PME, Warncke S, Gierlich J, Carell T: **Click-click-click: single to triple modification of DNA**. *Angew Chem Int Ed* 2008, **47**:3442-3444 http://dx.doi.org/10.1002/anie.200705664.

44. • Pfeiffer F, Tolle F, Rosenthal M, Brändle GM, Ewers J, Mayer G: **Identification and characterization of nucleobase-modified aptamers by click-SELEX**. *Nat Protoc* 2018, **13**:1153-1180 http://dx.doi.org/10.1038/nprot.2018.023.
This paper show the use of CuAAC postsynthetic modification of DNA in selection of modified aptamers.

45. Panattoni A, Pohl R, Hocek M: **Flexible alkyne-linked thymidine phosphoramidites and triphosphates for chemical or polymerase synthesis and fast postsynthetic DNA functionalization through copper-catalyzed alkyne-Azide 1,3-dipolar cycloaddition**. *Org Lett* 2018, **20**:3962-3965 http://dx.doi.org/10.1021/acs.orglett.8b01533.

46. Matyašovský J, Perlíková P, Malnuit V, Pohl R, Hocek M: **2-Substituted dATP derivatives as building blocks for polymerase-catalyzed synthesis of DNA modified in the minor groove**. *Angew Chem Int Ed* 2016, **55**:15856-15859 http://dx.doi.org/10.1002/anie.201609007.

47. Matyašovský J, Pohl R, Hocek M: **2-Allyl- and propargylamino-dATPs for site-specific enzymatic introduction of a single modification in the minor groove of DNA**. *Chem Eur J* 2018, **24**:14938-14941 http://dx.doi.org/10.1002/chem.201803973.

48. Balintová J, Špaček J, Pohl R, Brázdová M, Havran L, Fojta M, Hocek M: **Azidophenyl as a click-transformable redox label of DNA suitable for electrochemical detection of DNA–protein interactions**. *Chem Sci* 2015, **6**:575-587 http://dx.doi.org/10.1039/C4SC01906G.

49. Neef AB, Luedtke NW: **An azide-modified nucleoside for metabolic labeling of DNA**. *ChemBioChem* 2014, **15**:789-793 http://dx.doi.org/10.1002/cbic.201400037.

50. Merkel M, Peewasan K, Arndt S, Ploschik D, Wagenknecht H-A: **Copper-free postsynthetic labeling of nucleic acids by means of bioorthogonal reactions**. *ChemBioChem* 2015, **16**:1541-1553 http://dx.doi.org/10.1002/cbic.201500199.

51. Ren X, El-Sagheer AH, Brown T: **Efficient enzymatic synthesis and dual-colour fluorescent labelling of DNA probes using long chain azido-dUTP and BCN dyes**. *Nucleic Acids Res* 2016, **44**:e79 http://dx.doi.org/10.1093/nar/gkw028.

52. Ren X, El-Sagheer AH, Brown T: **Azide and trans-cyclooctene dUTPs: incorporation into DNA probes and fluorescent click-labelling**. *Analyst* 2015, **140**:2671-2678 http://dx.doi.org/10.1039/c5an00158g.

53. Abendroth F, Seitz O: **Double-clicking peptides onto phosphorothioate oligonucleotides: combining two proapoptotic agents in one molecule**. *Angew Chem Int Ed* 2014, **53**:10504-10509 http://dx.doi.org/10.1002/anie.201406674.

54. Eördögh Á, Steinmeyer J, Peewasan K, Schepers U, Wagenknecht HA, Kele P: **Polarity sensitive bioorthogonally applicable far-red emitting labels for postsynthetic nucleic acid labeling by copper-catalyzed and copper-free cycloaddition**. *Bioconjug Chem* 2016, **27**:457-464 http://dx.doi.org/10.1021/acs.bioconjchem.5b00557.

55. Gutsmiedl K, Fazio D, Carell T: **High-density DNA functionalization by a combination of Cu-catalyzed and cu-free click chemistry**. *Chem Eur J* 2010, **16**:6877-6883 http://dx.doi.org/10.1002/chem.201000363.

56. Borsenberger V, Howorka S: **Diene-modified nucleotides for the Diels-Alder-mediated functional tagging of DNA**. *Nucleic Acids Res* 2009, **37**:1477-1485 http://dx.doi.org/10.1093/nar/gkn1066.

57. Wu H, Devaraj NK: **Inverse electron-demand Diels-alder Bioorthogonal reactions**. *Top Curr Chem* 2016, **374**:3 http://dx.doi.org/10.1007/s41061-015-0005-z.

58. Schoch J, Wiessler M, Jäschke A: **Post-synthetic modification of DNA by inverse-electron-demand Diels-Alder reaction**. *J Am Chem Soc* 2010, **132**:8846-8847 http://dx.doi.org/10.1021/ja102871p.

59. Wang K, Wang D, Ji K, Chen W, Zheng Y, Dai C, Wang B: **Post-synthesis DNA modifications using a *trans*-cyclooctene click handle**. *Org Biomol Chem* 2015, **13**:909-915 http://dx.doi.org/10.1039/C4OB02031F.

60. •• Reisacher U, Ploschik D, Rönicke F, Cserép GB, Kele P, Wagenknecht H-A: **Copper-free dual labeling of DNA by triazines and cyclopropenes as minimal orthogonal *and* bioorthogonal functions**. *Chem Sci* 2019, **10**:4032-4037 http://dx.doi.org/10.1039/C8SC05588B.
This paper shows the first dual labelling of DNA with two orthogonal Cu-free click reactions.

61. Merkel M, Arndt S, Ploschik D, Cserép GB, Wenge U, Kele P, Wagenknecht H-A: **Scope and limitations of typical copper-free bioorthogonal reactions with DNA: reactive 2′-deoxyuridine triphosphates for postsynthetic labeling**. *J Org Chem* 2016, **81**:7527-7538 http://dx.doi.org/10.1021/acs.joc.6b01205.

62. Rieder U, Luedtke NW: **Alkene-tetrazine ligation for imaging cellular DNA**. *Angew Chem Int Ed* 2014, **53**:9168-9172 http://dx.doi.org/10.1002/anie.201403580.

63. Busskamp H, Batroff E, Niederwieser A, Abdel-Rahman OS, Winter RF, Wittmann V, Marx A: **Efficient labelling of enzymatically synthesized vinyl-modified DNA by an inverse-electron-demand Diels-Alder reaction**. *Chem Commun* 2014, **50**:10827-10829 http://dx.doi.org/10.1039/c4cc04332d.

64. Lehmann B, Wagenknecht HA: **Fluorogenic "photoclick" labelling of DNA using a Cy3 dye**. *Org Biomol Chem* 2018, **16**:7579-7582 http://dx.doi.org/10.1039/c8ob02068j.

65. Wu Y, Guo G, Yheng J, Xing D, Zhang T: **Fluorogenic "photoclick" labeling and Imaging of DNA with coumarin-fused tetrazole in vivo**. *ACS Sens* 2019, **4**:44-51 http://dx.doi.org/10.1021/acssensors.8b00565.

66. Dadová J, Orság P, Pohl R, Brázdová M, Fojta M, Hocek M: **Vinylsulfonamide and acrylamide modification of DNA for cross-linking with proteins**. *Angew Chem Int Ed* 2013, **52**:10515-10518 http://dx.doi.org/10.1002/anie.201303577.

67. Dadová J, Vrábel M, Adámik M, Brázdová M, Pohl R, Fojta M, Hocek M: **Azidopropylvinylsulfonamide as a new bifunctional click reagent for bioorthogonal conjugations: application for DNA-protein cross-linking**. *Chem Eur J* 2015, **21**:16091-16102 http://dx.doi.org/10.1002/chem.201502209.

68. Eberhard H, Diezmann F, Seitz O: **DNA as a molecular ruler: interrogation of a tandem SH2 domain with self-assembled, bivalent DNA-peptide complexes**. *Angew Chem Int Ed* 2011, **50**:4146-4150 http://dx.doi.org/10.1002/anie.201007593.

69. Weng L, Zhou C, Greenberg MM: **Probing interactions between lysine residues in histone tails and nucleosomal DNA via product and kinetic analysis**. *ACS Chem Biol* 2015, **10**:622-630 http://dx.doi.org/10.1021/cb500737y.

70. Olszewska A, Pohl R, Brázdová M, Fojta M, Hocek M: **Chloroacetamide-linked nucleotides and DNA for cross-linking with peptides and proteins**. *Bioconjug Chem* 2016, **27**:2089-2094 http://dx.doi.org/10.1021/acs.bioconjchem.6b00342.

71. Wu Q, Amrutkar SM, Shao F: **Sulfinate based selective labeling of 5-hydroxymethylcytosine: application to biotin pull down assay**. *Bioconjug Chem* 2018, **29**:245-249 http://dx.doi.org/10.1021/acs.bioconjchem.7b00826.

72. Diezmann F, Eberhard H, Seitz O: **Native chemical ligation in the synthesis of internally modified oligonucleotide-peptide conjugates**. *Biopolymers* 2010, **94**:397-404 http://dx.doi.org/10.1002/bip.21440.

73. Weisbrod SH, Marx A: **A nucleoside triphosphate for site-specific labelling of DNA by the Staudinger ligation**. *Chem Commun* 2007, **18**:1828-1830 http://dx.doi.org/10.1039/b618257g.

74. Lercher L, McGouran JF, Kessler BM, Schofield CJ, Davis BG: **DNA modification under mild conditions by Suzuki-Miyaura cross-coupling for the generation of functional probes**. *Angew Chem Int Ed* 2013, **52**:10553-10558 http://dx.doi.org/10.1002/anie.201304038.

75. Cahová H, Jäschke A: **Nucleoside-based diarzlethene photoswitches and theit facile incorporation into photoswitchable DNA**. *Angew Chem Int Ed* 2013, **52**:3186-3190 http://dx.doi.org/10.1002/anie.201209943.

76. Krause A, Hertl A, Muttach F, Jaschke A: **Phosphine-Free-Stille-Migita-chemistry for the mild and ortogonal modification of DNA and RNA**. *Chem Eur J* 2014, **20**:16613-16619 http://dx.doi.org/10.1002/chem.201404843.

77. Probst N, Lartia R, Théry O, Alami M, Defrancq E, Messaoudi S: **Efficient Buchwald-Hartwig-Migita cross-coupling for DNA thioglycoconjugation**. *Chem Eur J* 2018, **24**:1795-1800 http://dx.doi.org/10.1002/chem.201705371.

78. Winnacker M, Breeger S, Strasser R, Carell T: **Novel diazirine-containing DNA photoaffinity probes for the investigation of DNA-protein-interactions**. *ChemBioChem* 2009, **10**:109-118 http://dx.doi.org/10.1002/cbic.200800397.

79. •• Zawada Z, Tatar A, Mocilac P, Buděšínský M, Kraus T: **Transport of nucleoside triphosphates into cells by artificial molecular transporters**. *Angew Chem Int Ed* 2018, **57**:9891-9895 http://dx.doi.org/10.1002/anie.201801306.
This paper reports the transporter of dNTPs across the cell membrane. Using modified dNTPs it can lead to labelling of genomic DNA in living cells.

80. Ivancová I, Pohl R, Hubálek M, Hocek M: **Squaramate-modified nucleotides and DNA for specific cross-linking with Lys-containing peptides and proteins**. *Angew Chem Int Ed* 2019 . in press *https://doi.org/10.1002/anie.201906737*.

81. • Vaníková Z, Janoušková M, Kambová M, Krásný L, Hocek M: **Switching transcription with bacterial RNA polymerase through photocaging, photorelease and phosphorylation reactions in the major groove of DNA**. *Chem Sci* 2019, **10**:3937-3942 http://dx.doi.org/10.1039/c9sc00205g.
This paper reports the first switching (on and off) of transcription through reactions in the major groove of modified DNA.