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DNA Conjugation by Staudinger Ligation

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ABSTRACT

Two 5 modified 2'-deoxyuridin triphosphates and a 7 modified 2'-deoxy-7-deazaadenosine were synthesized carrying a terminal azide linked to the base. For probing the sterical influence on incorporation and Staudinger ligation different sized flexible linkers were chosen. All three nucleotides can completely replace their natural counterparts in primer extension as well as polymerase chain reactions (PCR) using *Pwo* DNA polymerase. For azide labeled primer extension products subsequent conjugation of suitably functionalized phosphines via Staudinger ligation was achieved, e.g. for the conjugation of biotin as an affinity tag.

INTRODUCTION

Linkage of a complex biomolecule with another molecular entity is often a challenging task. Albeit several bioconjugation methods are available, truly chemoselective methods are rarely represented. Recently, two reactions based on the specific reactivity of the azide moiety were introduced.[1,2] We concentrated on the Staudinger ligation to achieve bioconjugation on DNA. Developed by Bertozzi[3] this reaction has been applied recently for DNA labeling. Fluorescence labeling at the 5' terminus of DNA[4] and Cu(I) induced strand scission after methyltransferase mediated labeling of the DNA with subsequent labeling by Staudinger ligation has been achieved so far.[5] Here we present the generation of azide labeled DNA by azide modified triphosphates and DNA polymerase directed synthesis and the subsequent derivatisation by Staudinger ligation.

RESULTS AND DISCUSSION

First we synthesized different azide-modified nucleoside triphosphate building blocks to probe for the sterical influence on enzymatic incorporation and Staudinger ligation. Therefore differently sized flexible linkers were chosen for the adenosine[6] and thymidine[7] based types of dNTP analogues **1** to **3** (Fig. 1).

These dNTP analogues are readily incorporated into a growing DNA strand via a DNA polymerase directed DNA synthesis using different DNA polymerases such as *Taq*, Vent (exo$^-$) or *Pwo*. However, *Pwo* DNA polymerase was best suited for further investigations. Even the amplification of DNA using the modified dNTPs in a PCR

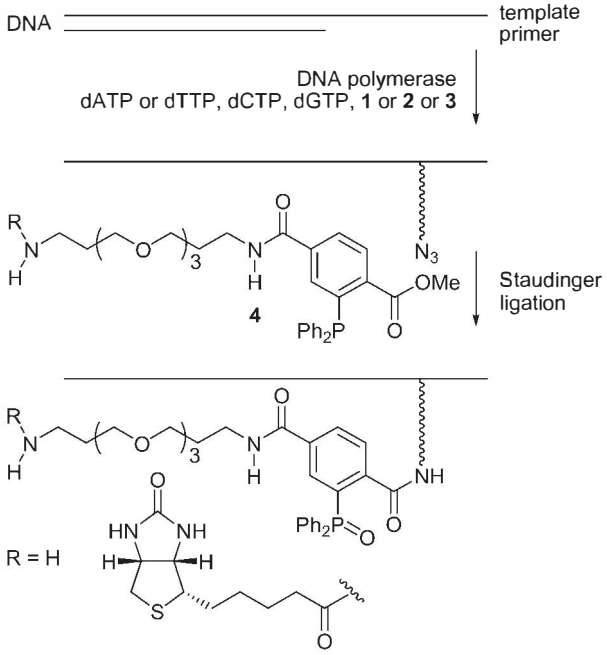

Fig. 2 Schematic depiction for post-synthetic labeling of DNA.

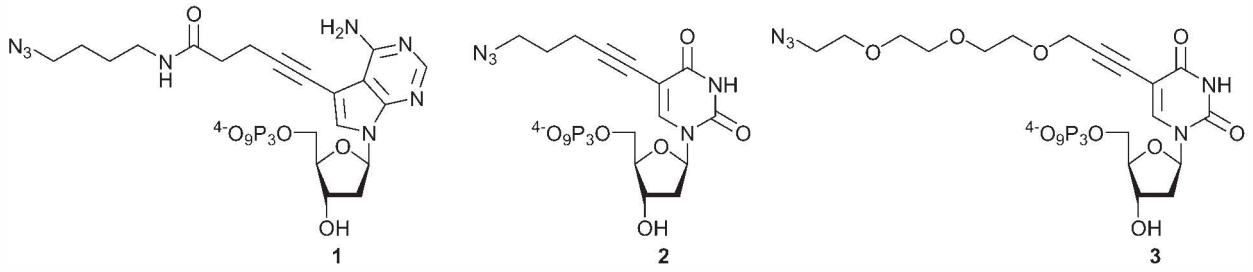

Fig. 1 Synthesized azide modified nucleoside triphosphates.

is possible. The synthesized DNA in turn can be conjugated using phosphines that bear several functional groups e.g. biotin (Fig. 2). Systematic investigations of organic co-solvent, temperature and reaction time revealed best conditions such as 60 °C, 12 h and DMF or ethanol as co-solvent. It turned out that the linker length thereby has only a marginal effect on incorporation of the triphosphates or subsequent Staudinger ligation.

The next synthesis step is now the derivatisation of the phospine moiety of **4** as coupling partner. Hitherto the triarylphosphineoxide remains in the conjugation product which may hamper subsequent investigations due to its steric demand. Traceless Staudinger ligation has been developed, where only an amide bond is formed as linkage in the conjugation product. Introducing water soluble functional groups and changing the ester from a benzoic acid ester to a phenol ester may allow traceless Staudinger ligation[8] without the need of an organic co-solvent. With the synthesis completed so far the synthesized phosphines have to be checked for their suitability for Staudinger ligation on DNA and optimal reaction conditions have to be found therefore.

CONCLUSION

The devised methods pave the way for efficient DNA conjugation under mild conditions just by adding the two reaction partners without requiring further reagents. Investigations to increase diversity of substrates and the coupling efficiency including traceless Staudinger ligation are underway.

REFERENCES

1. Breinbauer, R., Köhn, M. (2003) *ChemBioChem*, **4**, 1147-1149.
2. Köhn M., Breinbauer, R. (2004) *Angew. Chem. Int. Ed.*, **43**, 3106-3116.
3. Saxon, E., Bertozzi, C. R. (2000) *Science*, **287**, 2007-2010.
4. Wang, C. C., Seo, T. S., Li, Z., Ruparel, H. Ju, J. (2003) *Bioconjug. Chem.*, **14**, 697-701.
5. Comstock, L. R., Rajski, S. R. (2005) *J. Am. Chem. Soc.*, **127**, 14136-14137.
6. Weisbrod, S. H., Marx, A. (2007) *Chem. Commun.*, 1828-1830.
7. Baccaro, A., Weisbrod, S. H., Marx, A. (2007) *Synthesis*, 1949-1954.
8. Tam, A., Soellner, M. B., Raines, R.T. (2007) *J. Am. Chem. Soc.,* **129,** 11421-30.

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