Modular platform for modified aptamers

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1. Introduction

We have designed a modular platform for introducing new chemical modifications into single-stranded DNA (ssDNA) for its use as modified libraries for aptamers selection. Employing the "click-chemistry" (Copper-catalysed Azide-Alkyne Cycloaddition [1], CuAAC) we introduced a panel of chemical groups into a derivative of a deoxyuridine triphosphate and used it for synthesis of modified ssDNA which could later be "reverse transcribed" with a standard set of nucleotides (A, C, G, T) into unmodified DNA strand. Hence, we have arrived at **a modular method allowing to successfully introduce new chemical groups into the aptamers generation process**.

2. Rationale

The lack of chemical diversity of the four natural nucleotides limits aptamers versatility as binders and catalysts. Moreover, high susceptibility of natural nucleic acid-based aptamers to nuclease digestion restricts their use as therapeutic drugs and *in vivo* diagnostic agents. Similarily to other groups (see reviews [2,3]), we also searched for a way to introduce new chemical functionalities to DNA which would be compatible with the SELEX methodology. However, we focused on an approach capable of fulfilling certain requirements:

- one-step, robust chemical synthesis
- large variability possible
- one-step purification
- stability
- high polymerases compatibility

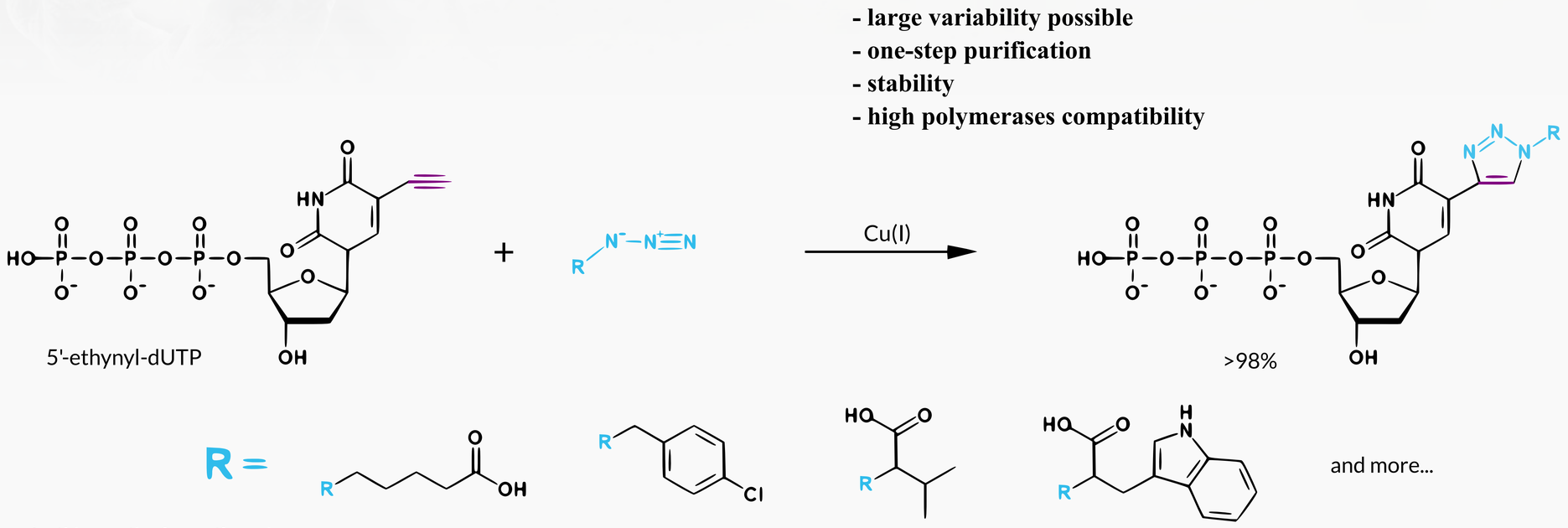

Figure 1. Modified nucleotides synthesis reaction scheme.

3. Materials & methods

3.1. Modified nucleotides synthesis

Using a commercially available compound - 5'-ethynyl-deoxyuridine triphosphate (EdUTP) as a substrate, we performed the CuAAC reaction with various azides to obtain chemically modified deoxyuracil analogues (Figure 1). Next, products were purified directly from the crude reaction solution via RP-HPLC using a C18 column, then lyophilized and dissolved again. Identity of the modified nucleotides was confirmed using LC/MS-TOF.

3.2. Enzymatic compatibility of new modified nucleotides

To confirm the incorporation of the modified nucleotides into ssDNA, Primer EXtension reaction (PEX) was performed, previously optimized for the chosen polymerase (Deep Vent (exo-)) and various reaction conditions (e.g. timing scheme). Modified ssDNA was PAGE-purified and used as a template in a "reverse transcription" PEX with four natural nucleotides to verify its SELEX compatibility.

4. Results

4.1. Modified nucleotides synthesis

All of 11 tested modifying azides gave distinct products (Figure 1) with expected masses as assessed by LC/MS-TOF. Exemplary purification RP-HPLC chromatogram is shown in Figure 2 (compared with the chromatogram for the substrate EdUTP), and a corresponding MS-TOF mass spectrum in Figure 3. CuAAC reactions yields were quantitative - no EdUTP could be seen during the HPLC purification.

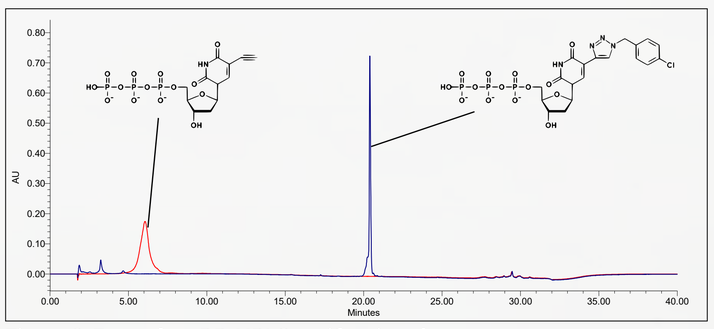

Figure 2. Exemplary RP-HPLC purification chromatogram
The red line represents a negative control, where the CuAAC reaction was not initiated (no cuprum source added). The blue chromatogram represents the purification of one of the modified nucleotides; no substrate is seen confirming quantitative yield. Inset: corresponding structures of the substrate EdUTP (left) and the modified nucleotides (right).

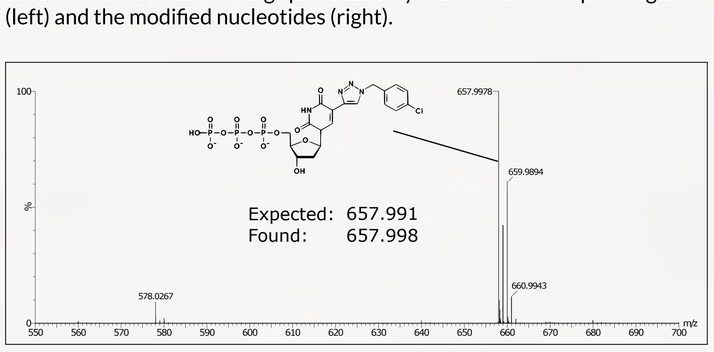

Figure 3. MS-TOF analysis of a modified nucleotide triphosphate
The main isotopic peak is identified. The inset represents the corresponding structure.

4.2. Enzymatic compatibility of new modified nucleotides

For all 11 modified nucleotides, we observed medium to high efficiency of their incorporation into ssDNA during PEX with Deep Vent (exo-) polymerase (staining of oligonucleotides varies depending on the modification carried; Figure 4A). Four polymerases were tested in various conditions (Figure 4B-C), and Deep Vent (exo-) was chosen for further tests. This polymerase was highly capable of reconstructing unmodified ssDNA in a "reverse transcription" PEX using four natural nucleotides and a template bearing one of the modified nucleotides instead of every thymidine (Figure 4D).

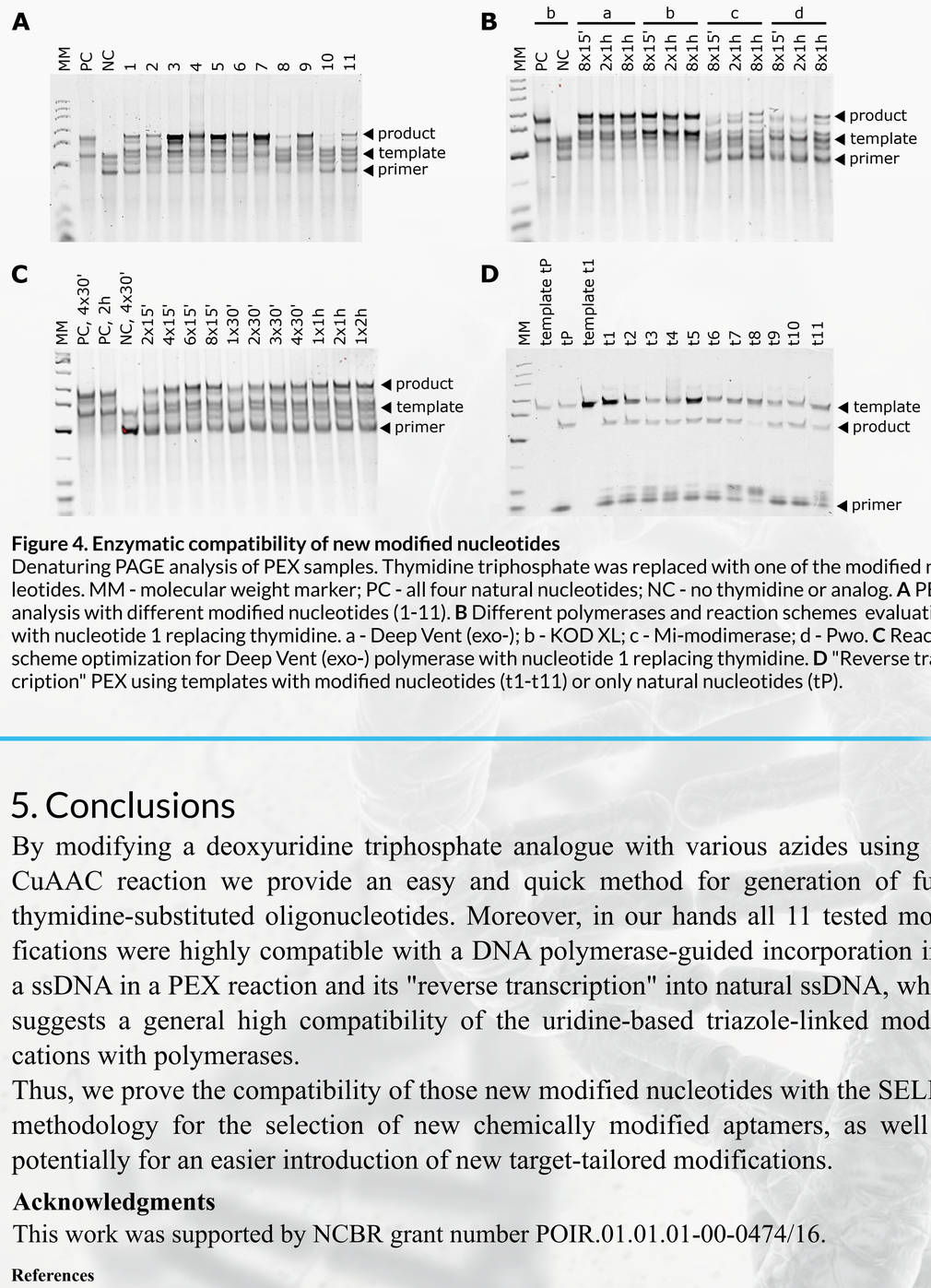

Figure 4. Enzymatic compatibility of new modified nucleotides
Denaturing PAGE analysis of PEX samples. Thymidine triphosphate was replaced with one of the modified nucleotides. MM - molecular weight marker; PC - all four natural nucleotides; NC - no thymidine or analog. **A** PEX analysis with different modified nucleotides (1-11). **B** Different polymerases and reaction schemes evaluation with nucleotide 1 replacing thymidine. a - Deep Vent (exo-); b - KOD XL; c - Mi-modimerase; d - Pwo. **C** Reaction scheme optimization for Deep Vent (exo-) polymerase with nucleotide 1 replacing thymidine. **D** "Reverse transcription" PEX using templates with modified nucleotides (t1-t11) or only natural nucleotides (tP).

5. Conclusions

By modifying a deoxyuridine triphosphate analogue with various azides using the CuAAC reaction we provide an easy and quick method for generation of fully thymidine-substituted oligonucleotides. Moreover, in our hands all 11 tested modifications were highly compatible with a DNA polymerase-guided incorporation into a ssDNA in a PEX reaction and its "reverse transcription" into natural ssDNA, which suggests a general high compatibility of the uridine-based triazole-linked modifications with polymerases.

Thus, we prove the compatibility of those new modified nucleotides with the SELEX methodology for the selection of new chemically modified aptamers, as well as potentially for an easier introduction of new target-tailored modifications.

Acknowledgments

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References

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