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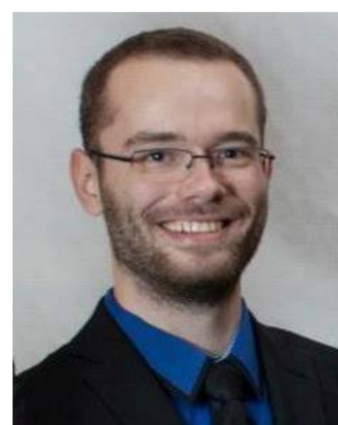
2-Substituted dATP Derivatives as Building Blocks for Polymerase-Catalyzed Synthesis of DNA Modified in the Minor Groove



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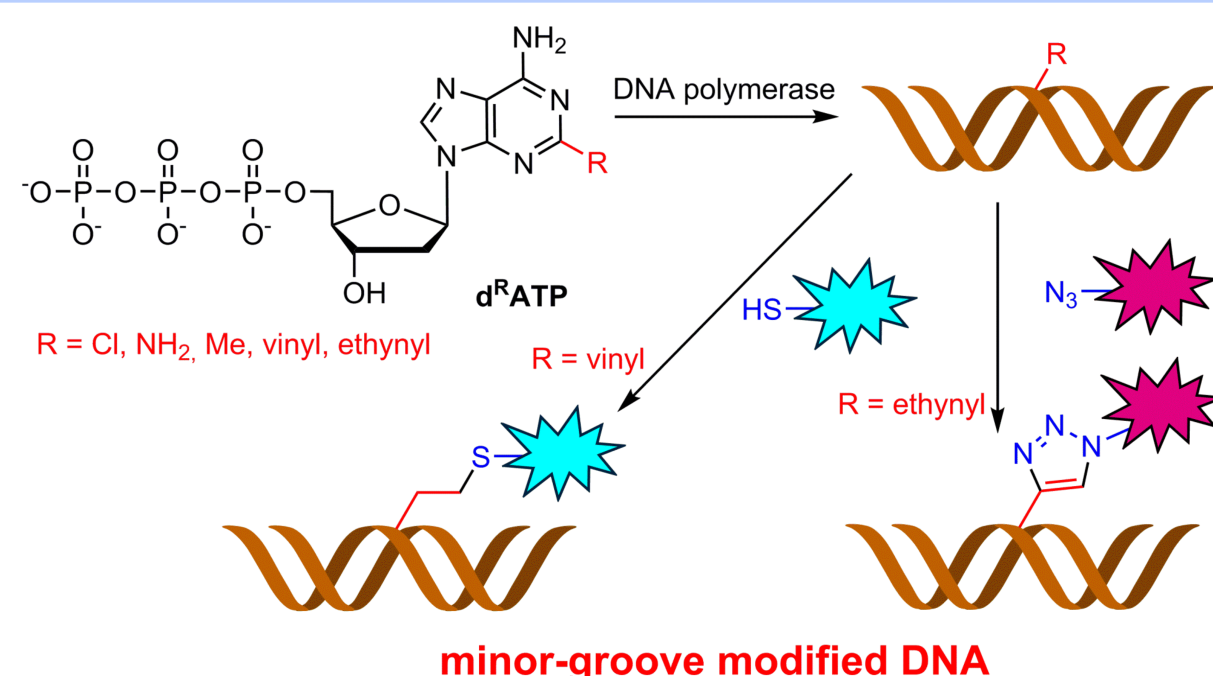
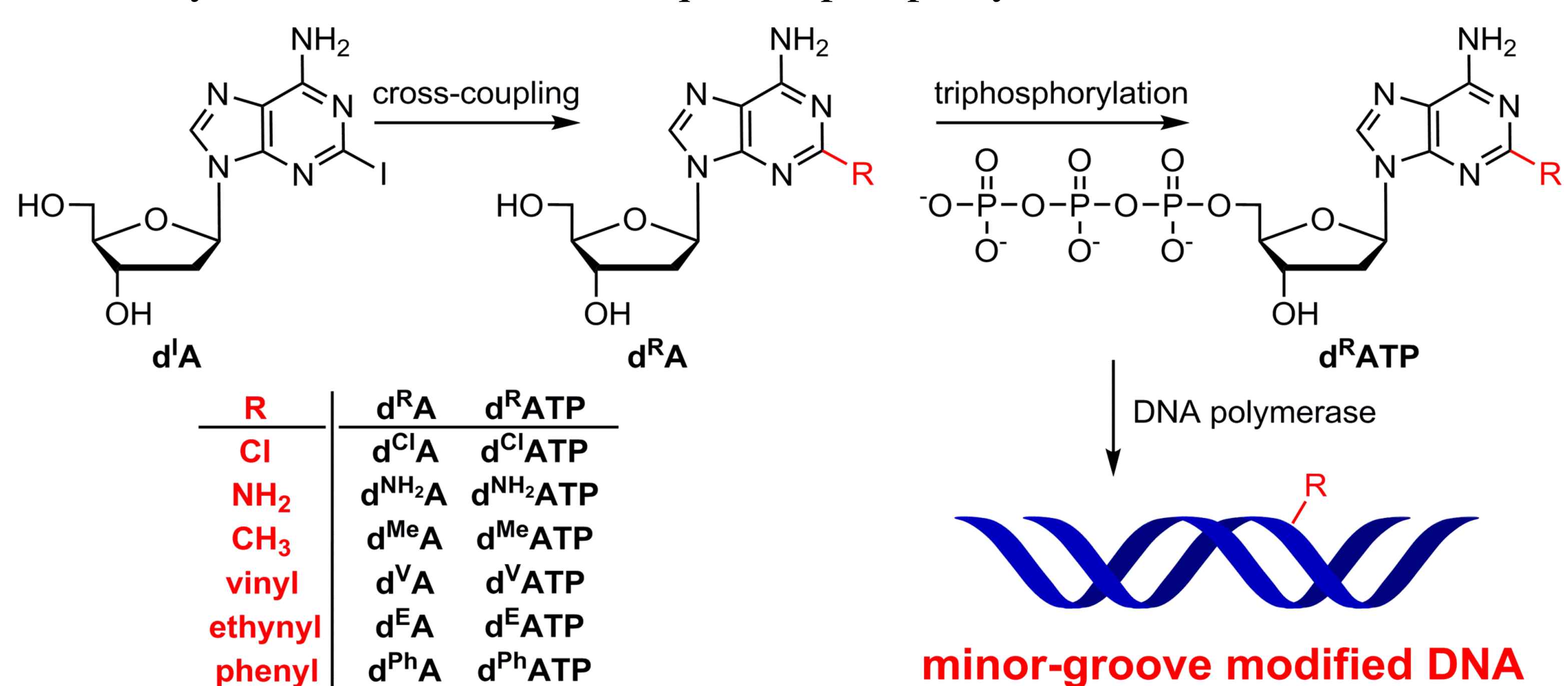
Introduction

Enzymatic synthesis of functionalized DNA, using base-modified 2'-deoxyribonucleoside triphosphates (dNTPs), is a widely used tool with potential applications in chemical biology, bioanalysis or diagnostics.[1] The modification is usually attached to the position 5 of pyrimidines or position 7 of 7-deazapurines. This ensures that the modification is pointing out into the major groove of DNA, not destabilizing the duplex. Moreover, corresponding dNTPs are likely good substrates for DNA polymerases and therefore can be used for enzymatic synthesis of modified oligonucleotides (ONs).[2] The only minor-groove base-modified nucleotides, that were reported as good substrates for DNA polymerases, were 2-chloroadenine and 2,6-diaminopurine dNTPs.[3,4] Therefore we envisaged that a small substituent at position 2 of purine heterocycle may not fully disturb the key Watson-Crick H-bonding, nor the important minor groove interactions necessary for chain extension by DNA polymerase.[5] Herein we report the first example of enzymatic synthesis of minor-groove base-modified DNA.[6]



Synthesis of 2-modified dATP derivatives

To study the effect of bulkiness of the substituent on incorporation by DNA polymerases, a series of six 2-modified dATP derivatives bearing Cl, NH$_2$, CH$_3$, vinyl, ethynyl and phenyl (**d^{R}ATPs**) was designed and synthesized. Since **d^{Cl}ATP** and **d^{NH_2}ATP** were known,[4,5] synthesis of the rest of the series involved cross-coupling reactions of key 2-iodo-2'-deoxyadenosine **d^{I}A** and subsequent triphosphorylation.



Scheme 1: Synthesis of 2-modified dATP derivatives and their use in enzymatic synthesis of minor-groove modified DNA

Post-synthetic modification of DNA minor groove with fluorescent labels utilizing click chemistry

DNAs modified in the minor groove with one or four vinyl groups (**DNA1^{V}A** and **DNA4^{V}A**) or with one or four ethynyl groups (**DNA1^{E}A** and **DNA4^{E}A**) were subjected to post-synthetic modification using click chemistry. Vinyl group underwent thiol-ene reaction, whereas ethynyl group was envisaged for CuAAC reaction. Thiol-ene reactions of vinyl modified DNA with fluorescent coumarinemethyl thiol **CM-SH** for 3 days at 37 °C gave ca. 60% conversions (based on PAGE analysis, Figure 2d), providing blue-fluorescent conjugates (Figure 2a, 2c). CuAAC reaction of ethynyl modified DNA with Cy3-N$_3$ proceeded smoothly (Figure 2f) giving red-fluorescent products (Figure 2b, 2e).

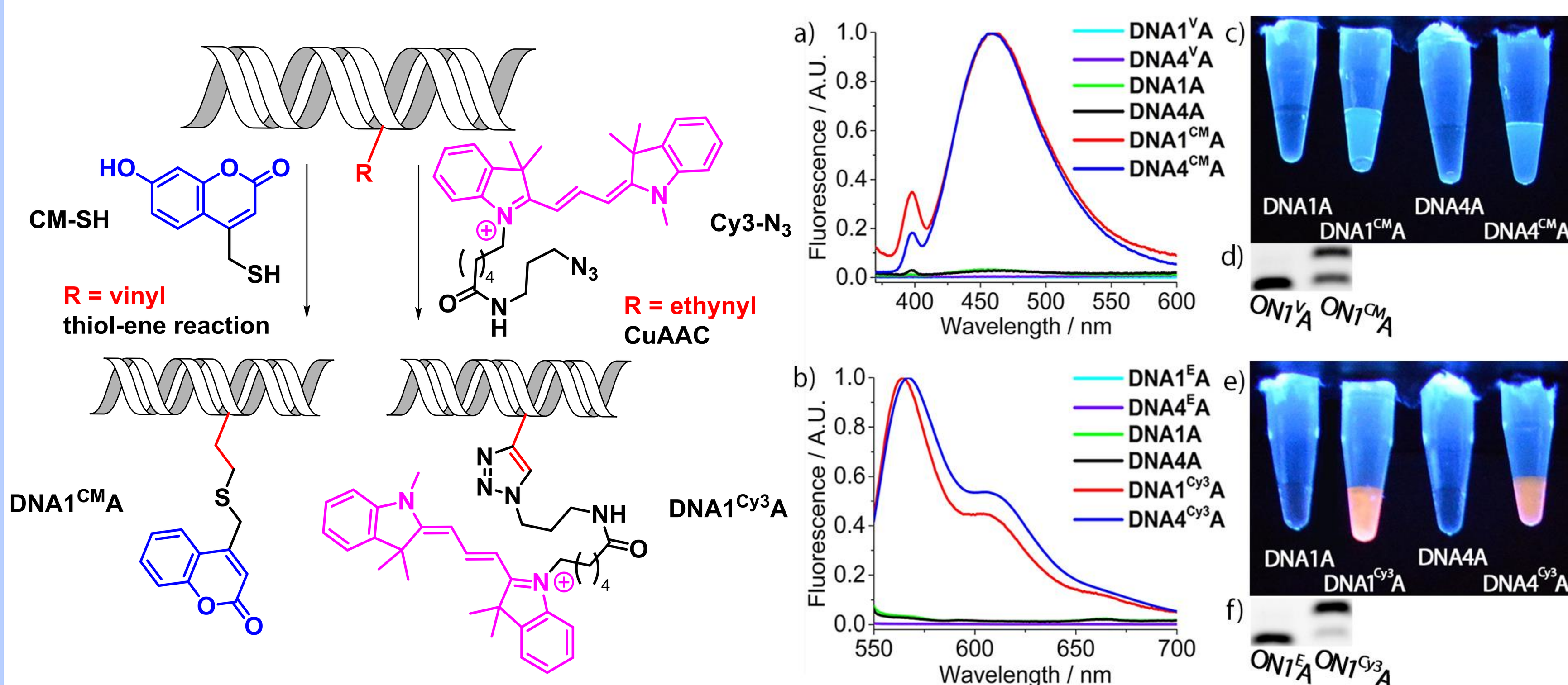


Figure 2: Analysis of post-synthetic labeling of minor-groove modified DNA by fluorescent labels using click chemistry

Incorporation of d^{R}ATPs into DNA by PEX with KOD XL DNA polymerase

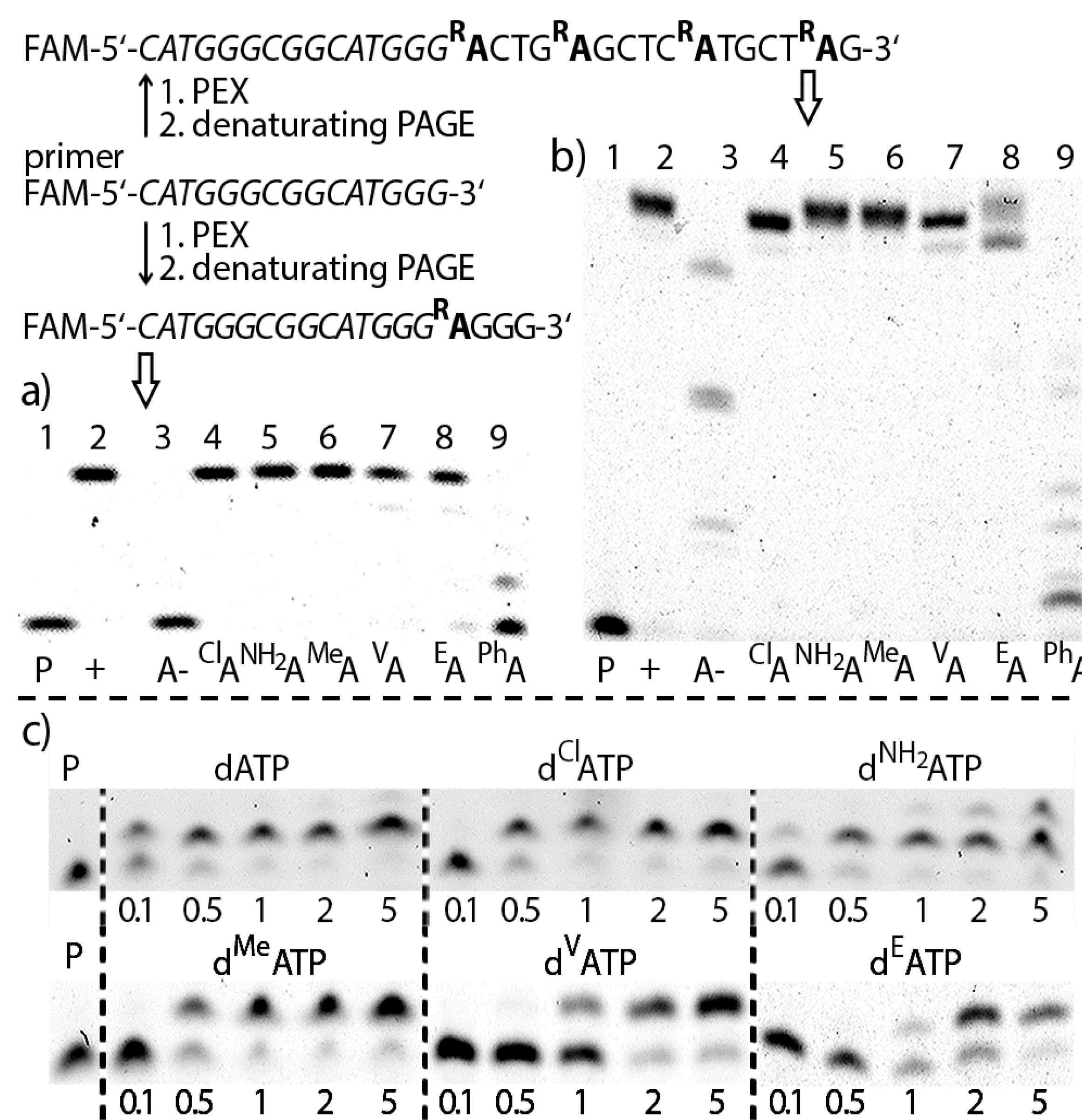


Figure 1: PAGE analysis of PEX products

Prepared **d^{R}ATPs** were then tested as substrates for DNA polymerases in primer extension (PEX). Products of PEX with KOD XL DNA polymerase with either one modification (**DNA1^{R}As**, Figure 1a) or four modifications (**DNA4^{R}As**, Figure 1b), were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE). All tested **d^{R}ATPs** were good substrates for KOD XL DNA polymerase, with the exception of **d^{Ph}ATP**, which is not a substrate for DNA polymerase, since almost no extension was observed. A simple kinetic analysis (Figure 1c) compared the rates of incorporation of the modified **d^{R}ATPs** with natural dATP. The rates of incorporation of smaller **d^{Cl}ATP**, **d^{NH_2}ATP** and **d^{Me}ATP** was comparable to the natural dATP, whereas bigger **d^{V}ATP** and **d^{E}ATP** took approximately 2 min to reach completion.

Denaturating temperatures of minor-groove modified DNA

DNA	T_m / °C	ΔT_m / °C	DNA	T_m / °C	ΔT_m / °C
DNA1A	72.2	–	**DNA4A**	79.4	–
DNA1^{Cl}A	69.6	– 2.6	**DNA4^{Cl}A**	71.0	– 2.1
DNA1^{NH_2}A	72.5	+ 0.3	**DNA4^{NH_2}A**	81.4	+ 0.5
DNA1^{Me}A	70.5	– 1.7	**DNA4^{Me}A**	72.3	– 1.8
DNA1^{V}A	66.6	– 5.6	**DNA4^{V}A**	72.6	– 1.7
DNA1^{E}A	66.1	– 6.1	**DNA4^{E}A**	71.1	– 2.1
DNA1^{Cy3}A	69.0	– 3.2			

Table 1: Denaturing temperatures of modified DNA duplexes

Denaturing temperatures of all PEX products show that, with the exception of 2,6-diaminopurine, that stabilizes the DNA thanks to additional H-bond with T, all the other modifications destabilize the duplexes.

Conclusion

In conclusion, we found out that dATP derivatives modified with a small substituent at position 2 (Cl, NH$_2$, CH$_3$, vinyl and ethynyl) are all substrates for DNA polymerases and were used for enzymatic synthesis of minor groove modified DNA, while **d^{Ph}ATP** was already too bulky to be incorporated. Vinyl and ethynyl minor-groove modified DNA can be post-synthetically modified by thiol-ene or CuAAC reactions, which was shown by fluorescent labelling. Recently, a new study showed that N^2-substituted 2'-deoxyguanosine triphosphates act as selective substrates for Human DNA polymerase κ.[7]

References:

[1] Nakatani, K.; Tor, Y. (Eds) *Modified Nucleic Acids*, *Nucleic Acids and Molecular Biology Series*, Vol. 31, Springer 2016, pp. 1–276.
[2] a) Hocek, M. *J. Org. Chem.* **2014**, *79*, 9914–9921; b) Hottin, A.; Marx, A. *Acc. Chem. Res.* **2016**, *49*, 418–427; c) Hollenstein, M. *Molecules* **2012**, *17*, 13569–13591.
[3] Parker, W. B.; Shaddix, S. C.; Chang, C.-H.; White, E. L.; Rose, L. M.; Brockman, R. W. ; Shortnacy, A. T.; Montgomery, J. A.; Secrist III, J. A.; Bennett, L. L. *Cancer Res*. **1991**, *51*, 2386–2394
[4] a) Chollet, A.; Kawashima, E. *Nucleic Acids Res.* **1988**, *16*, 305–317; b) Kutyavin, I. V. *Biochemistry* **2008**, *47*, 13666–13673.
[5] Matsuda, S.; Leconte, A. M.; Romesberg, F. E. *J. Am. Chem. Soc.* **2007**, *129*, 5551–5557.
[6] Matyašovský, J.; Perlíková, P.: Malnuit, V.; Pohl, R.; Hocek, M. *Angew. Chem. Int. Ed.* **2016**, *55*, 15856–15859.
[7] Gowda, A. S. P.; Lee, M.; Spratt, T. E. *Angew. Chem. Int. Ed.* **2017**, *56*, 2628–2631.

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