Competitive primer extension of base-modified dNTPs in the presence of natural dNTPs.

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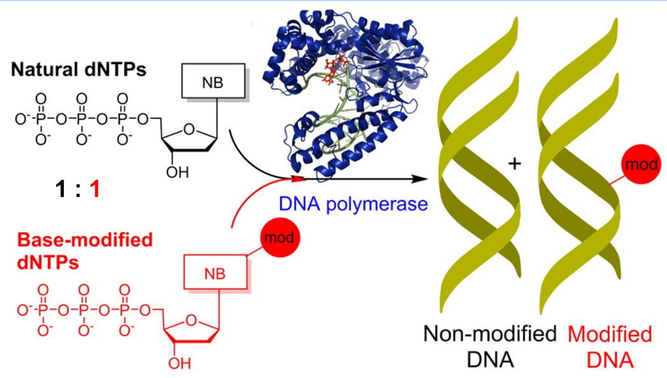
Introduction

Competitive incorporations of some 7-substituted-7-deazaadenine and 5-substituted cytosine 2'-deoxynucleosides in the presence of natural dNTPs were previously studied in our group. Results showed that most 7-aryl-7-deaza dA^{R}TP are better substrates for most polymerases than natural dATP.[1] We have now extended this work to a comprehensive study of competitive PEX and PCR, including different dN^{R}TPs of all four bases in different ratios with the natural dNTPs. This systematic study showed that most modified dN^{R}TPs containing π-electron modifications are excellent substrates and are generally better incorporated into DNA than natural dNTPs.[2] As kinetic studies and semiempirical calculations confirmed, the affinity of the modified dN^{R}TPs bearing the π-electron modifications for the active site of the polymerase complex with the primer and template is enhanced because of increased π-stacking, explaining, thus, their preferential incorporation by DNA polymerases with respect to their natural counterparts.[2]



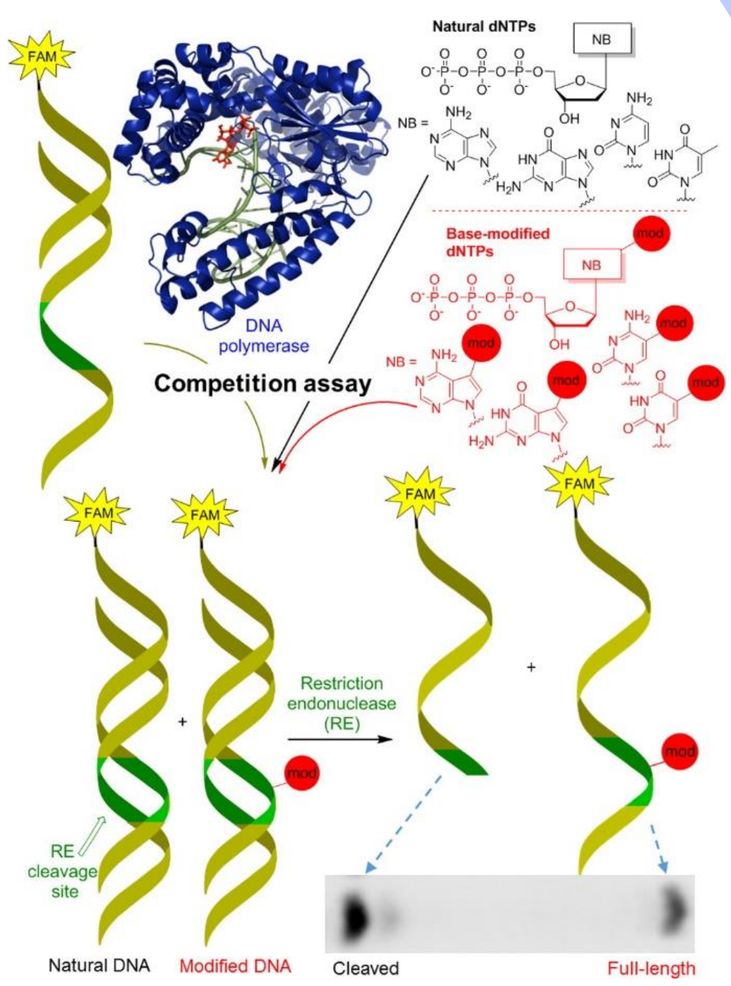
Principle of competitive incorporations

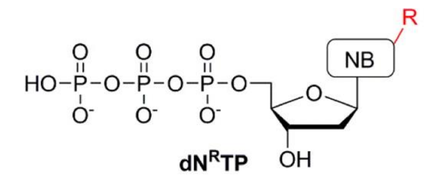
An approach based on cleavage by restriction endonucleases (REs) was employed to test the competitive incorporation of modified dNTPs toward natural dNTPs.

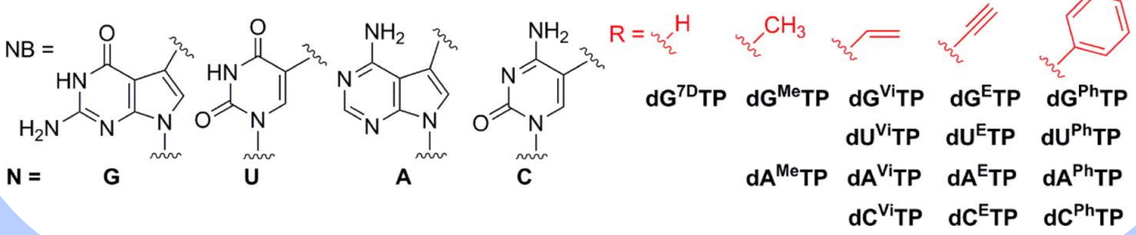
Some REs are very sensitive to base modifications in their recognition sequence.[3]

Therefore, PEX can be performed using a 1:1 mixture of modified and natural dNTPs, followed by cleavage by the appropriate RE and finally PAGE analysis.

DNA chains containing the modified dNTP are not cleaved by the selected RE. The ratio of modified and unmodified dNTP incorporated can thus be quantified by comparing the intensity of the two spots corresponding to the products.[4,5]

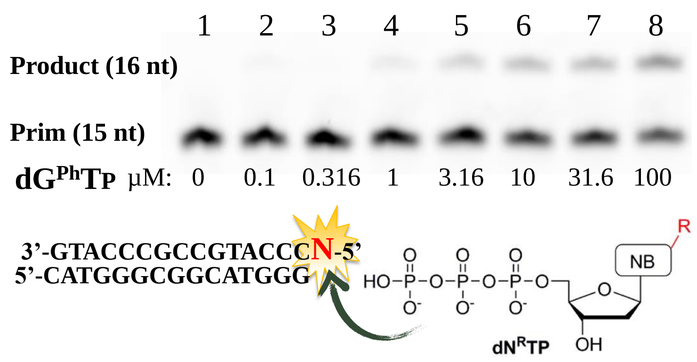






Kinetics of single incorporation

	K_M	k_{cat}	Ratio[a]
dGTP	5.3	13.8	1
dG^{Ph}TP	4.1	13.7	**1.4**
TTP	3.2	6.6	1
dU^{Ph}TP	7.9	9.3	0.6
dATP	8.7	8.0	1
dA^{Ph}TP	5.6	13.5	**2.5**
dCTP	6.7	6.1	1
dC^{Vi}TP	14.1	15.4	**1.2**
dC^{Ph}TP	6	7.5	**1.4**



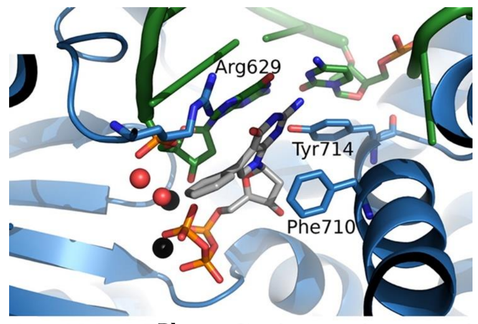
a) **Ratio** *is the* ***discrimination rate*** *and is defined as* $(k_{cat}/K_M)_{Modif}/(k_{cat}/K_M)_{natural}$

While no significant differences were found in the k_{cat} values of the modified with respect to the ones of non-modified dNTPs, 7-phenyl-substituted 7-deazapurine nucleotides (dG^{Ph}TP and dA^{Ph}TP) showed the highest discrimination rate (Ratio, in the table). These results indicate the higher affinity of 7-phenyl-substituted 7-deazapurine for the active site of the complex of polymerase with primer and template and are in perfect agreements withthose found in the competition experiments.

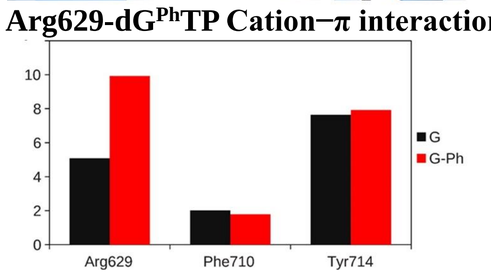
Molecular Modelling

Scores of the Natural dNTPs and dN^{Ph}TPs

dNTP	Score	Relative score
dGTP	−108.5	
dG^{Ph}TP	**−129.0**	**−20.5**
dUTP	−72.4	
TTP	−70.5	
dU^{Ph}TP	−61.8	10.5 (to UTP), 8.7 (to TTP)
dATP	−86.9	
dA^{Ph}TP	−97.4	**−10.5**
dCTP	−68.4	
dC^{Ph}TP	−70.2	1.8



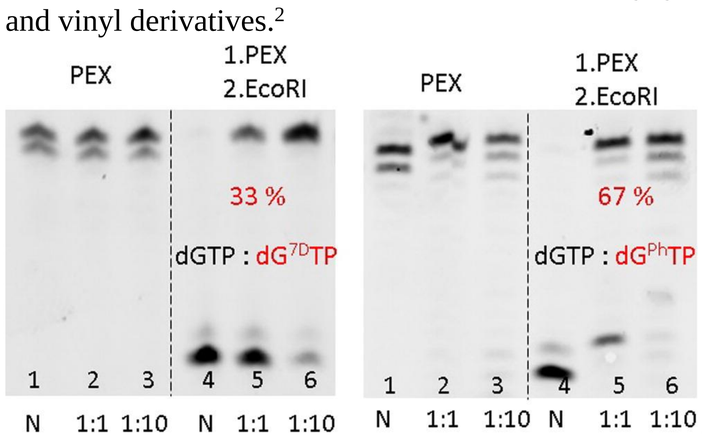
Arg629-dG^{Ph}TP Cation−π interaction



The 7-phenyl-7-deazapurine nucleotides had larger affinity than the native dGTP or dATP. The score of dC^{Ph}TP was only slightly more negative than that of dCTP whereas the modified dU^{Ph}TP was computed to have a lower affinity than the natural TTP. The virtual glycine scan showed that the increase in the binding is largely due to the cation−π interaction of Arg629 with the phenyl group of dG^{Ph}TP.[2]

Competitions using Bst DNA polymerase

Generally, 7-substituted-7-deazapurine dNTPs were found to be better substrates than 5-modified dUTP and dCTP for Bst DNA polymerase. Interestingly, most of the 7-modified 7-deaza dGTPs and dATPs, were accepted by DNA polymerases better than natural dGTP and dATP respectively. The Bst large fragment DNA polymerase showed the highest affinity for 7-phenyl-7-deaza purine triphosphates. In general, despite their size, the phenyl bearing dNTPs were found to be better substrates than the smaller ethynyl and vinyl derivatives.[2]



	Competition[a]
dG^{7D}TP	**33**
dG^{Me}TP	**42**
dG^{Vi}TP	**39**
dG^{E}TP	**58**
dG^{Ph}TP	**67**
dU^{Vi}TP	**41**
dU^{E}TP	**28**
dU^{Ph}TP	**57**
dA^{Me}TP	**35**
dA^{Vi}TP	**70**
dA^{E}TP	**72**
dA^{Ph}TP	**76**
dC^{Vi}TP	**49**
dC^{E}TP	**49**
dC^{Ph}TP	**52**

a) **Competition** *is defined as the % of functionalized DNA prepared in PEX with mix of dN^{X}TP:dNTP in 1:1 ratio.*

Conclusion

Our systematic study revealed that most dN^{R}TPs were good to excellent substrates for Bst polymerase. 7-Deazapurine dNTPs bearing π-electron-containing substituents (ethynyl and phenyl, as well as 7-vinyl-7-deazaadenine) are generally even better substrates of Bst polymerase than natural dATP or dGTP, respectively. Similar results have been recently found also for other 7-akyne- or 7-alkene-modified 7-deazaadenines as substrates of KlenTaq DNA polymerase.[6] 5-Substituted dC^{R}TPs are comparable to dCTP, whereas the 5-substituted dU^{R}TPs are generally worse substrates than TTP. The measured kinetic parameters follow the same trend and confirm that 7-phenyl-7-deazapurine dNTPs have higher affinity to the active site of the polymerase than their natural counterparts.

References:

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