Investigating D-*carba*-dT Triphosphate in Primer-Extension Assays

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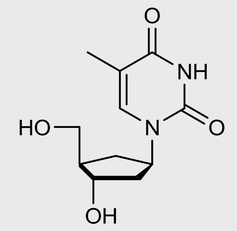
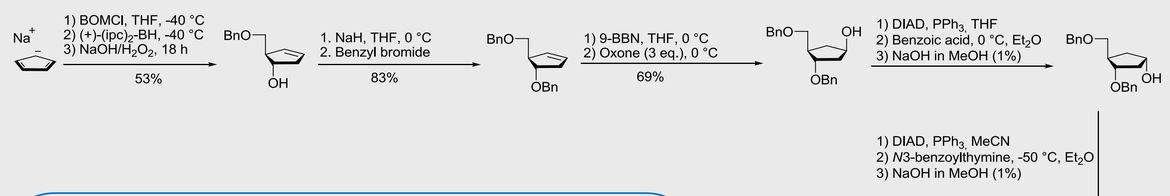
Introduction

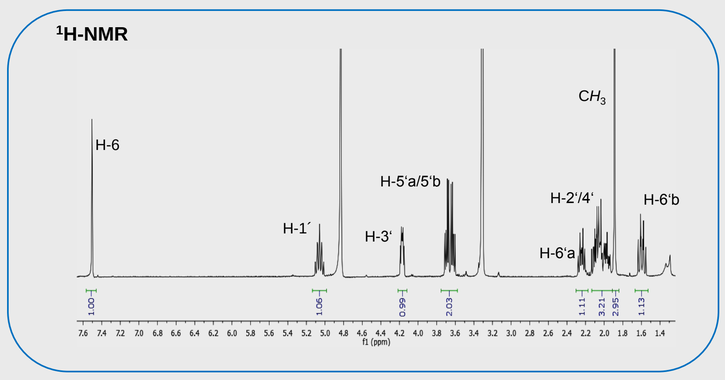
Carbocyclic nucleoside analogues are extremely attractive compounds in drug development due to their high biological activity against several viruses. Apart from their antiviral activity, the carbocyclic compounds benefit from their higher stability against enzymatic degradation due to the replacement of the oxygen atom by a methylene group. This replacement leads to increased lipophilicity as well as increased bioavailability. The carbocyclic nucleoside analogue D-*carba*-dT showed promising antiviral activities against diverse viruses, e.g. HIV-1, HIV-2 and VV.[1] The antiviral activity of D-*carba*-dT may be the result of the flexibility of the cyclopentane ring. Due to this flexibility it can be assumed that D-*carba*-dT can adopt a conformation that makes the compound to be a substrate for intracellular kinases and polymerases.[2]



Synthesis of D-*carba*-dT

The convergent synthesis was a suitable method for the formation of a variety of nucleoside analogues. The synthesis of D-*carba*-dT required the formation of several stereogenic centers, which can be achieved in a six-step synthesis.[1,3]

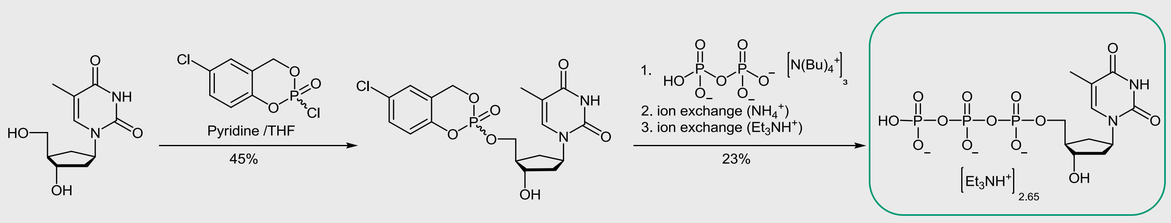


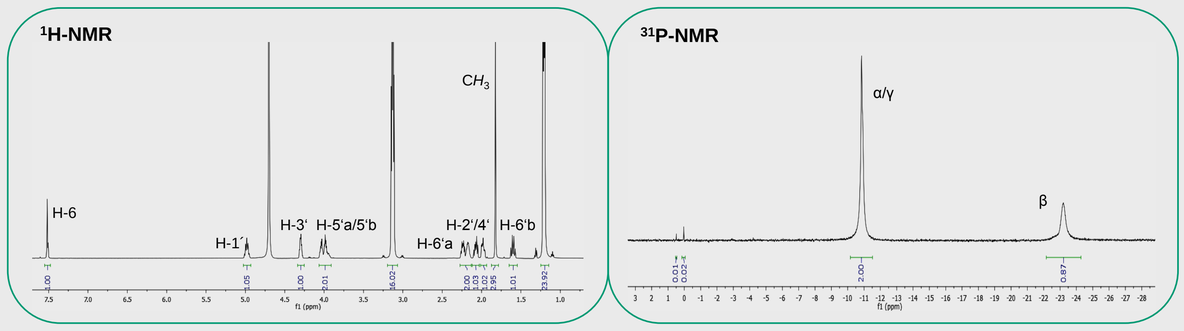


Objectives

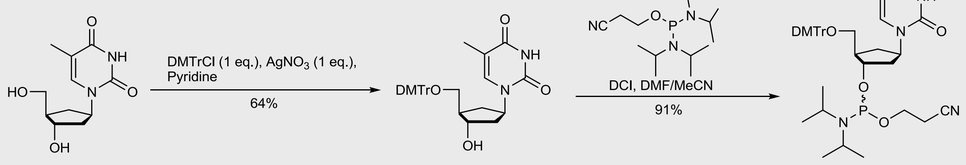
Since detailed information about the chain termination processes of D-*carba*-dT are still uncertain, the aim of this project was to perform primer-extension assays to gain insights in the mode of action of the human DNA polymerases β and γ using D-*carba*-dT triphosphate. Additionally, studies in bypassing the “lesion” caused by D-*carba*-dT in oligonucleotides were examined. Furthermore, also possible point mutations were investigated by performing these primer-extension assays.

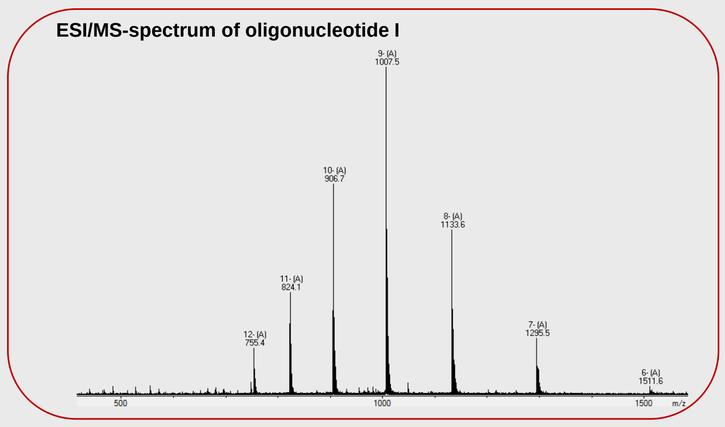
Synthesis of D-*carba*-dT Triphosphate





Synthesis of oligonucleotides containing D-*carba*-dT





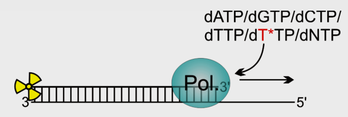
I: 3´-GCA ACC AGG ACT TCC TCC TAT CCA AT*T TAA-5´

II: 3´-GCA ACC AGG ACT TCC TCC TAT CCA AT*T* TAA-5´

III: 3´-GCA ACC AGG ACT TCC TCC TAT CCA ATT* TAA-5´

Biochemical studies

A: Examination of D-*carba*-dTTP (dT*TP) in primer-extension assays by human DNA polymerases β and γ



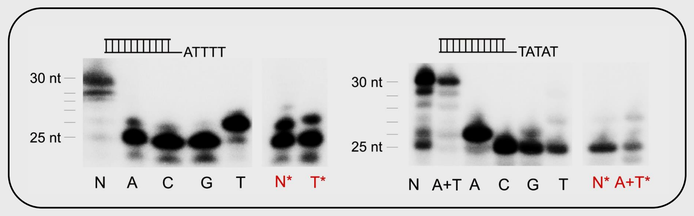
Assay: Polymerase 0.25-0.05 u, dNTPs [12.5 µM]

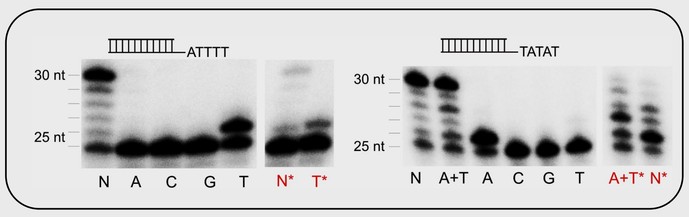
Conditions: 37 °C, 15-30 min.

Human DNA polymerase γ

1. After incorporation of dT*TP no complete elongation of the growing DNA-strands was detected.

→ „delayed chain termination vs. immediate chain termination“

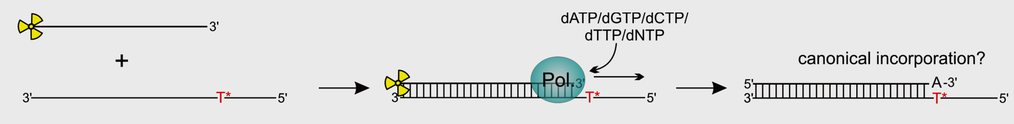




Human DNA polymerase β

1. After incorporation of dT*TP there is a slower, but still complete elongation of the growing DNA strand observable.

B: Examination of incorporated D-*carba*-dT in oligonucleotides in primer-extension assays by Klenow fragment, human DNA polymerases β and γ

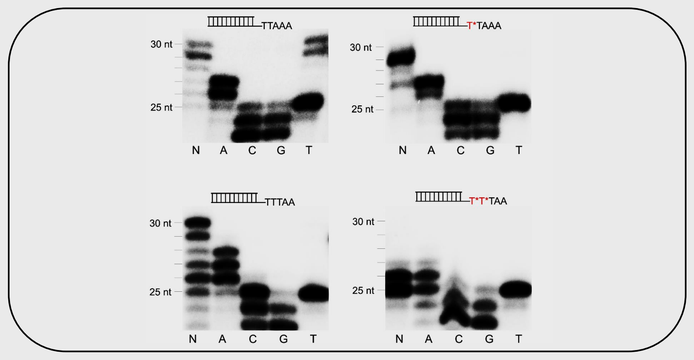


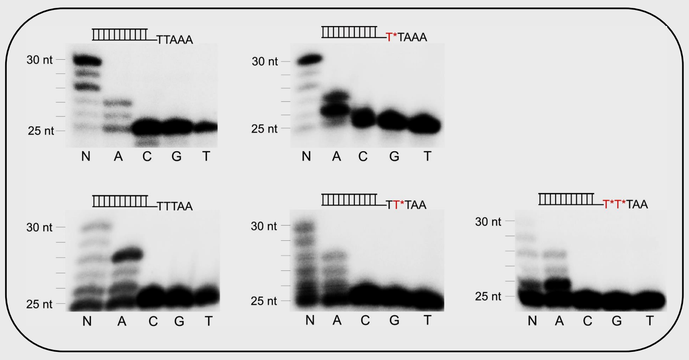
Assay: Polymerase 0.25-0.05 u, dNTPs [12.5 µM].

Conditions: 37 °C, 15-30 min.

Using the Klenow fragment

1. Single incorporation of D-*carba*-dT into oligonucleotide led to termination of elongation after four nucleotides.
2. The presence of two consecutive D-*carba*-dT‘s in the oligonucleotide caused an immediate termination.



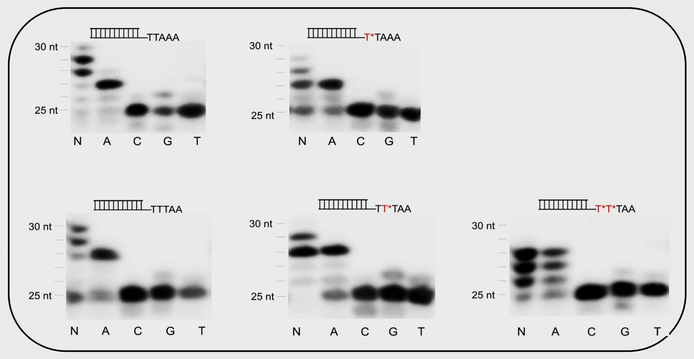


Human DNA polymerase β

1. The efficiency of the elongation of the DNA strand depended on the position in which D-*carba*-dT was incorporated into the oligonucleotides.
2. The consecutive incorporation of two D-*carba*-dT´s resulted in a weaker elongation.

Human DNA polymerase γ

1. In contrast to human DNA-polymerase β, no dependency was observed with respect to the position in which D-*carba*-dT was incorporated in the oligonucleotides.
2. Consecutive incorporation of two D-*carba*-dT´s led to termination after incorporation of one further nucleotide.



Conclusion

After the synthesis of the triphosphate-form of D-*carba*-dT and the incorporation of D-*carba*-dT in the oligonucleotides, primer-extension assays were performed using the human DNA polymerase β and γ. The human DNA polymerase β and γ reveal in the primer-extension assays with D-*carba*-dTTP the tendency to the unique “delayed chain termination” mechanism, although it is more pronounced in the case of the human DNA polymerase β (Biochem. studies A).

The successfully synthesized oligonucleotides reveal no termination of the elongation of the growing DNA strands using the human DNA polymerase β. In contrast, utilizing the oligonucleotide with two consecutively incorporated D-*carba*-dT’s and the human DNA polymerase γ, an incomplete elongation occurred (Biochem. studies B). Additionally, no point mutations were detected in the case of D-*carba*-dT modified oligonucleotides.

References

[1] M. Mahler, B. Reichardt, P. Hartjen, J. van Lunzen, C. Meier, *Eur. J. Org. Chem*. **2012**, 11046-11062. **[2]** P. Boyer, B. Vu, Z. Ambrose, J. G. Julias, S. Warnecke, C. Liao, C. Meier, V. E. Marquez, S. H. Hughes, *J. Med. Chem.* **2009**, *52*, 5356-5364. **[3]** S. Jessel, C. Meier, *Eur. J. Org. Chem*. **2011**, 1702-1713.

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