Synthesis, Characterization and Effects on DNA-Polymerases of C8-Arylamine-modified 2´-dG-5´-Triphosphates

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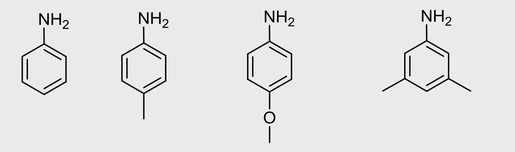
Introduction

Monocyclic aromatic amines **1-4** are found in the environment and belong to the class of borderline carcinogens. After metabolic activation they form covalent DNA adducts suspected to induce chemical carcinogenesis.[1] Among DNA-damages 8-(*N*-acetyl-*N*-arylamino)-2'-dG and 8-(*N*-arylamino)-2´-dG are predominately found adducts.[2] The *N*-acetyl group seems to plays a key role as the two C8-modified nucleosides were reported to adopt different conformations of the glycosidic bond. While 8-(*N*-arylamino)-2´-dG favors the *anti*-conformation, the acetylated nucleoside prefers *syn*-conformation and therefore differ in their physicochemical and biological properties.

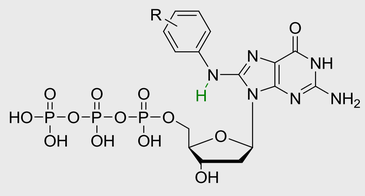


1 (**An**) Aniline | **2** (**Tol**) Toluidine | **3** (**Anis**) Anisidine | **4** (**DMA**) 3,5-Dimethylaniline

C8-Arylamine Modifications

Lesion-bearing DNA-strands and nucleosides have been extensively studied. However, little is known about lesion-bearing triphosphates. Therefore, non-acetylated 8-(*N*-arylamino)-2´-deoxyguanosine-5´-triphosphate (C8-*N*H-dG*TP) **5** and 8-(*N*-acetyl-*N*-arylamino)-2´-deoxyguanosine-5´-triphosphate (C8-*N*Ac-dG*TP) **6** were synthesized using the *cyclo*Sal-approach. The following issues were objectives of this project:

- determination of the glycosidic conformation compared to their modified nucleoside counterparts
- synthesis of site-specific modified DNA-strands by different polymerases
- to get insights into polymerase substrate specificity



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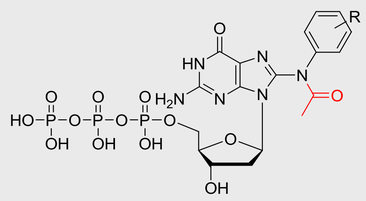
anti-conformation ?



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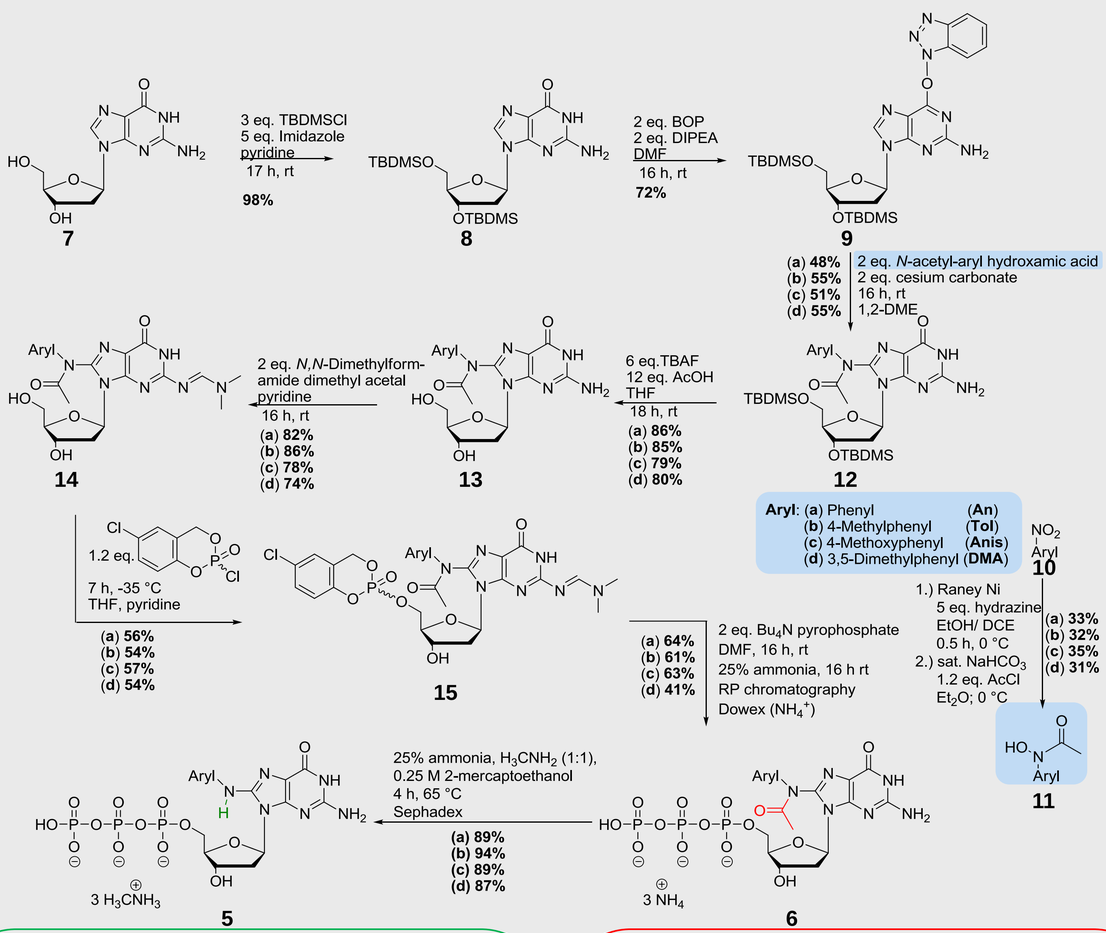
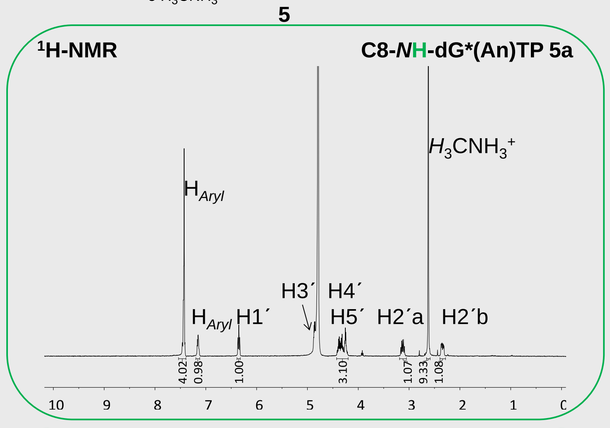
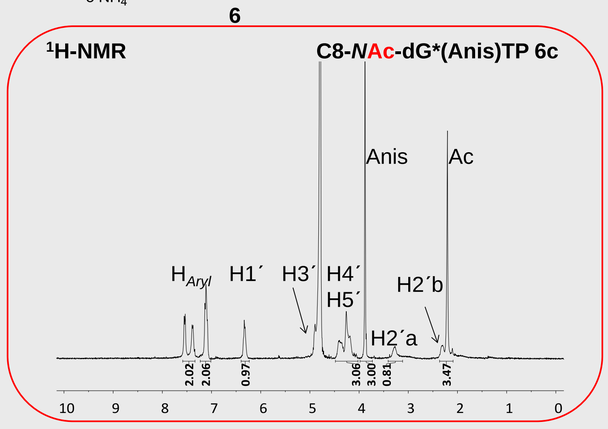
syn-conformation ?

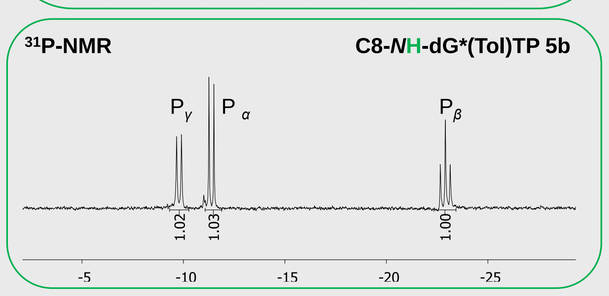
Synthesis

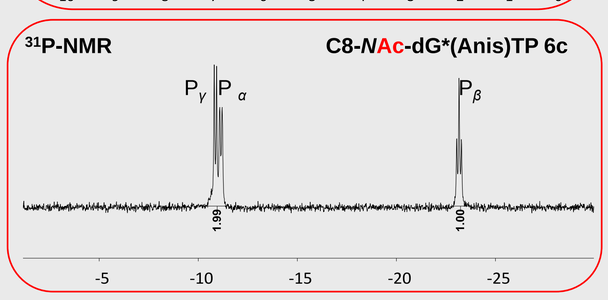
The *cyclo*Sal-approach was applied to the synthesis of 8-(arylamino)-modified 2´-dG nucleotides.[3-5]



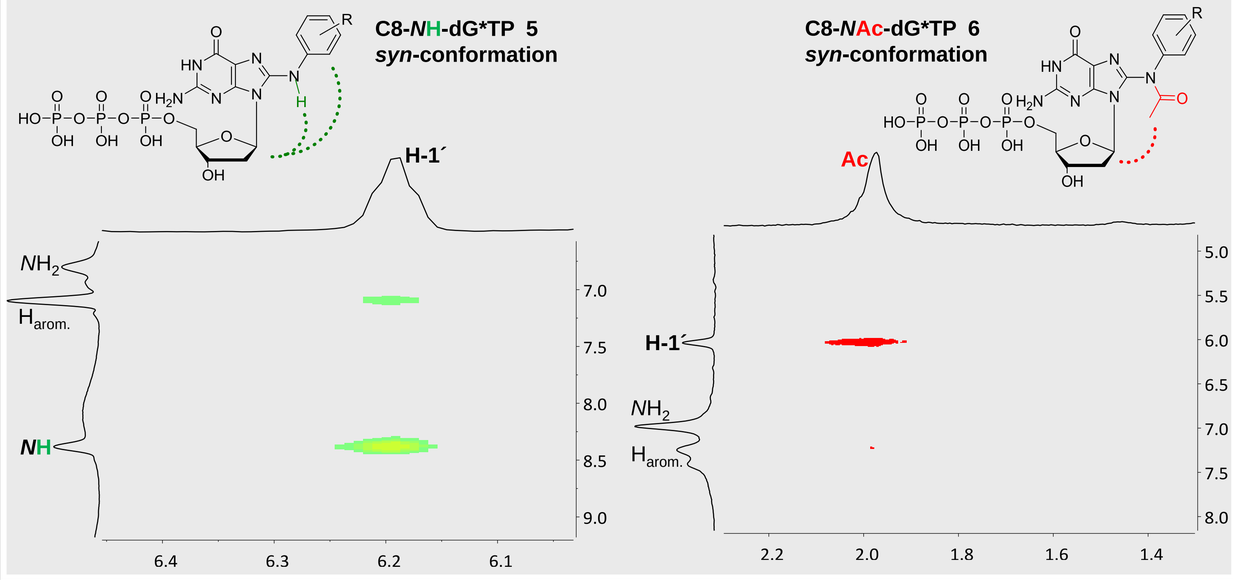




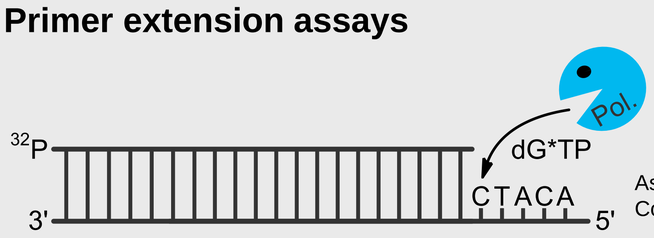




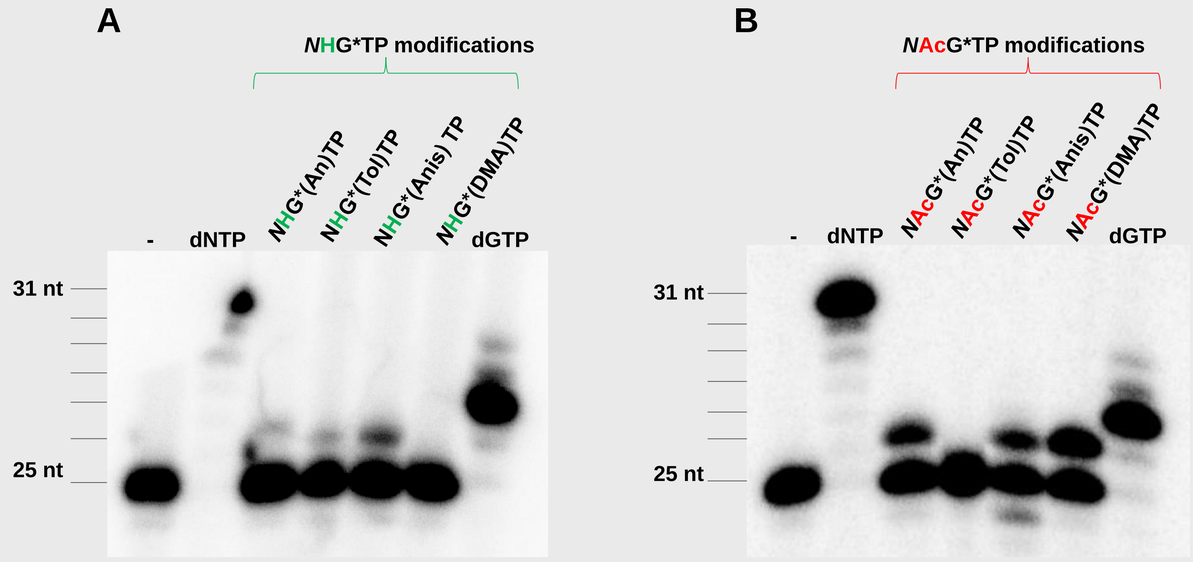
NOESY studies



Primer extension assays



Assay: Template [0.25 pmol], 2.5 U KF exo-, dG*TP [20 µM]
Conditions: 37 °C, 5 min.



Autoradiograms of representative denaturing (8 M urea) 15% polyacrylamide gels.

Both types of lesions, the non-acetylated *N*HG*TPs **A** and the *N*-acetylated *N*AcG*TPs **B**, were incorporated by Klenow Fragment exo- but to a small extent.

Conclusion

⇒ Surprisingly, both non-acetylated C8-*N*H-dG*TPs **5** and C8-*N*Ac-dG*TPs **6** adopted the *syn*-conformation in contrast to their modified nucleoside counterparts which was proven by NOESY spectroscopy

⇒ In primer extension assays the incorporation of both types of modifications C8-*N*H-dG*TPs and C8-*N*-Ac-dG*TPs was observed

⇒ However, lesion-bearing triphosphates were incorporated to a small extent only which makes the synthesis of site-specific modified DNA-strands difficult

⇒ The *syn*-conformation of both non-acetylated and acetylated triphosphates shows that ***no*** correlation can be made from lesion-bearing nucleosides to nucleotides or even DNA.

References

[1] Frederick, C. B., Mays, J. B., Ziegeler, D. M., Guengerich, F. P., Kadlubar, F. F., *J. Cancer Res. Clin. Oncol.* **1986**, *112*, 100-106. **[2]** Beland, F. A., Beranek, D. T., Dooley, K. L., Heflich, R. H., Kadlubar, F. F., *Environ. Health Perp*. **1983**, *49*, 125-134. **[3]** Krüger, S., Meier, C., *Eur. J. Org*. *Chem*. **2013**, *6*, 1158-1169. **[4]** Warnecke, S., Meier, C., *J. Org. Chem*. **2009**, *74*, 3024–3030. **[5]** Tonn, V. C., Meier, C., *Chem. Eur. J*. **2011**, *17*, 9832-9842.