# Cell Uptake of Di*PP*ro- and Tri*PPP*ro-Nucleotides by employing fluorescent Nucleoside Analogs

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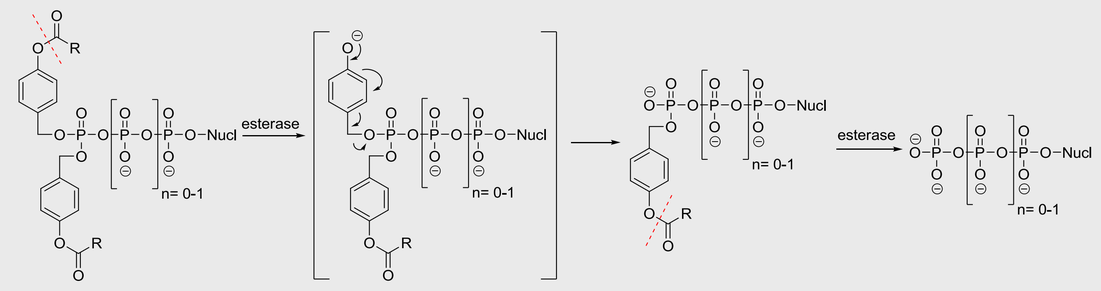
## Introduction

Several pronucleotide concepts have been developed to improve the antiviral activity in comparison to the parent nucleosides. The active form of the nucleosides is predominantly the corresponding triphosphate, which is generated intracellular by cellular kinases. Due to substrate specificity of the involved kinases this metabolism is often inefficient and applying the drug as its nucleoside triphosphate would be reasonable. With the Di*PP*ro-[1] and Tri*PPP*ro-approach[2,3], recently developed, nucleoside di- and triphosphates were bioreversibly masked. Hydrolysis studies in CEM/0 cell extracts and in vitro antiviral evaluation of these prodrugs showed promising results.[1-3]

## Concept

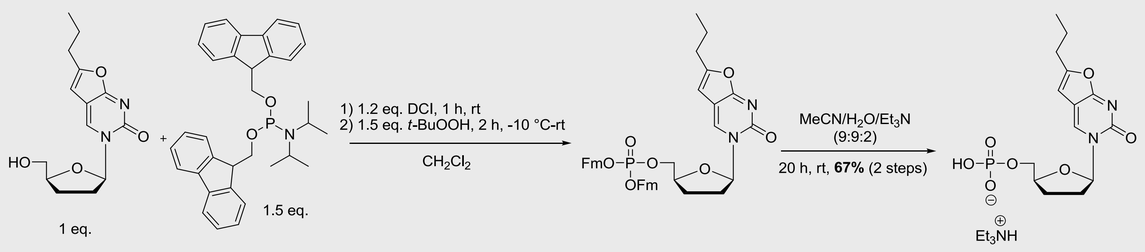
To enable the diffusion through the cell membrane of the phosphorylated nucleoside, two acyloxybenzyl esters are linked to the β- or γ-phosphate group. The intracellular cleavage of the masking units of the prodrugs is achieved by enzymatic hydrolysis of the acyl ester followed by rapid 1,6-elimination, so the nucleoside di- or triphosphate is released.



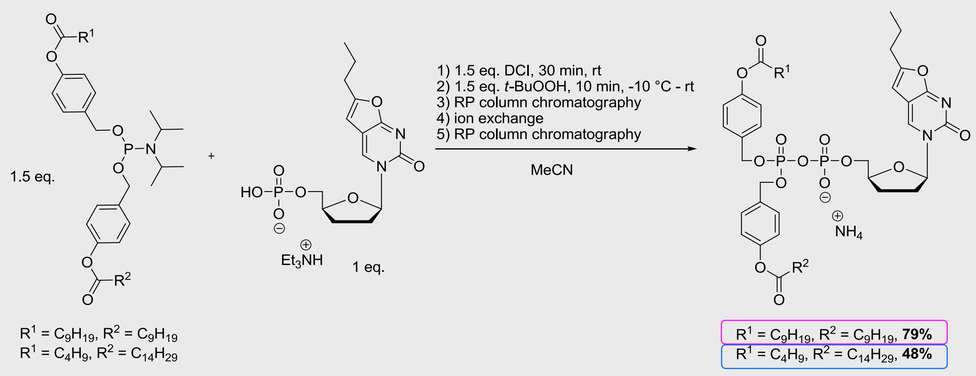

## Objectives

One option for further studies for validation of the concept is the use of fluorescent prodrug analogs. Therefore, a fluorescent probe with only minor structural changes compared to anti-HIV drugs like d4T is needed. Bicyclic nucleoside analogs (BCNAs) fulfill these requirements. In addition, BCNAs are not substrates for cellular kinases.[4] So, if phosphorylated BCNA-species could be detected in cell uptake studies this would strongly indicate the successful uptake of the compound and their desired enzymatic hydrolysis.

## Synthesis of the Di*PP*ro-ddBCNADP Prodrugs

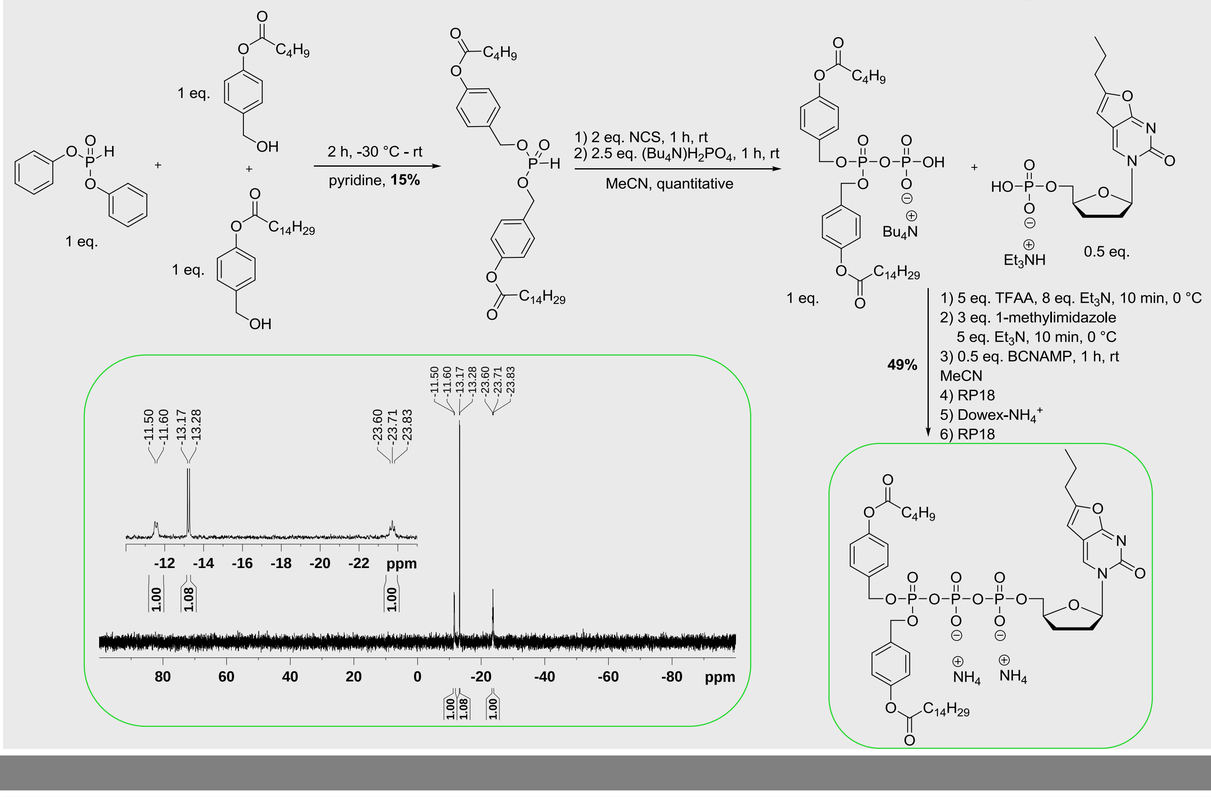



- Sowa-Ouchi method unsuccessful because of the acid lability of the nucleoside
- *cyclo*Sal technique unsuccessful due to purification problems

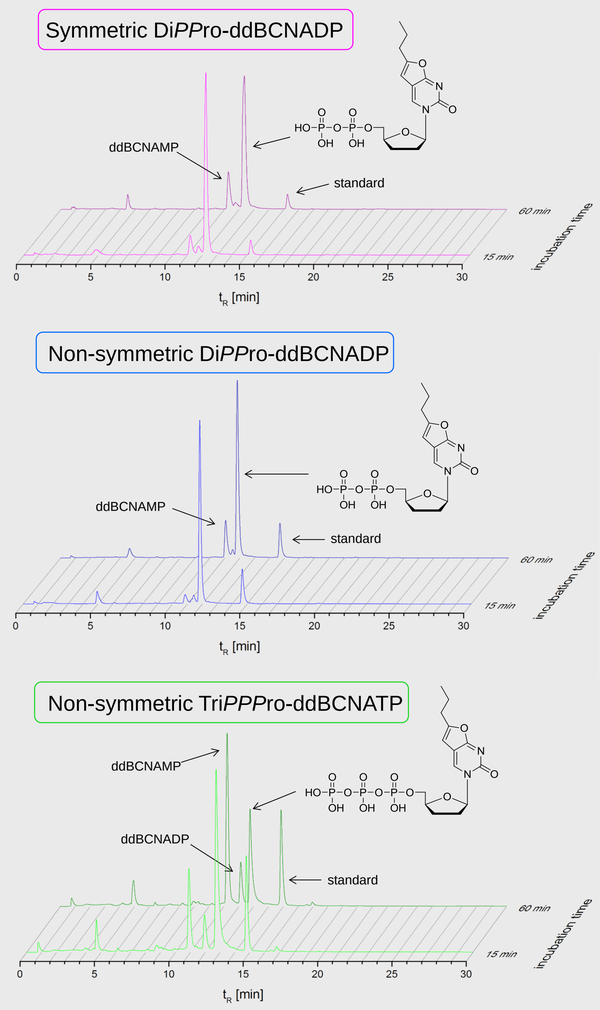



R¹ = $C_9H_{19}$, R² = $C_9H_{19}$, **79%**

## Synthesis of the non-symmetric Tri*PPP*ro-ddBCNATP Prodrug




## Cell Uptake of the Di*PP*ro- and Tri*PPP*ro-Nucleotides

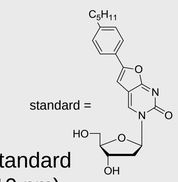



Di*PP*ro-ddBCNADP

- Detection of ddBCNADP and -MP
- Main metabolite was the diphosphate
- Ratio of the detected metabolites was in the same range
- In case of the symmetric prodrug a 2.5-fold higher amount of ddBCNA-compounds in the cells in comparison to the non-symmetric prodrug was detected

Tri*PPP*ro-ddBCNATP

- Detection of ddBCNATP, -DP and -MP
- Main metabolite after 15 minutes was the triphosphate
- With regard to the results of the non-symmetric Di*PP*ro-prodrug, an unexpected high amount of MP was observed.




After incubation of the CEM cells with a solution of 10 µM of the respective compound and 0.2 µM of the standard in RPMI medium the lysate was analyzed by RP-HPLC with fluorescence detection ($\lambda_{ex}$ = 340 nm, $\lambda_{em}$ = 410 nm).

## Conclusion and Outlook

Successfully, fluorescently labelled Di*PP*ro- and Tri*PPP*ro-nucleotides were synthesized. The cell uptake studies with these prodrugs confirmed the successful formation of the nucleoside di- and triphosphate in the cell, as already suggested by preliminary experiments in our group.[4] So, this prodrug approach can bypass the rate-limiting phosphorylation steps by cellular kinases and could thus lead to higher antiviral activity in comparison to the parent nucleoside. Now, further modifications of the masking unit of the prodrugs should be examined with this assay to study their uptake and metabolism in cell environment.

## References

[1] T. Schulz, J. Balzarini, C. Meier, *ChemMedChem* **2014**, *9*, 762-775. [2] T. Gollnest, T. Dinis de Oliveira, D. Schols, J. Balzarini, C. Meier, *Nat. Commun.* **2015**, *6*, 8716. [3] T. Gollnest, T. Dinis de Oliveira, A. Rath, I. Hauber, D. Schols, J. Balzarini, C. Meier, *Angew. Chem. Int. Ed.* **2016**, *55*, Ahead of Print. [4] R. Sienaert, L. Naesens, A. Brancale, E. De Clercq, C. McGuigan, J. Balzarini, *Mol. Pharmacol.* **2002**, *61*, 249-254.

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