The Di*PP*ro Approach: New Insights in Diphosphate Prodrugs

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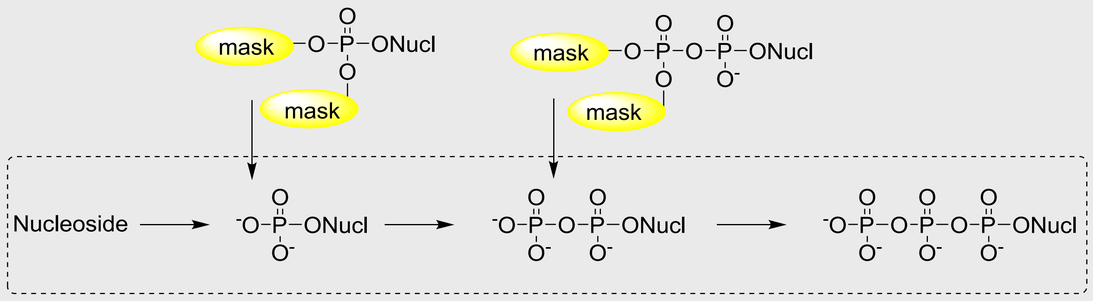
Introduction

Nucleoside analogues are extensively applied as agents in antiviral and antitumor therapy. Their activity depends on their bioactivation by kinases. The metabolism leads via the monophosphate and the diphosphate to the active triphosphate. However, in the case of nucleoside analogues cellular kinases often catalyze this metabolism insufficiently.[1] The result is a loss of antiviral activity. The use of prodrugs can circumvent limitations in single steps within this metabolism by bypassing the involved phosphorylating enzymes.[2]

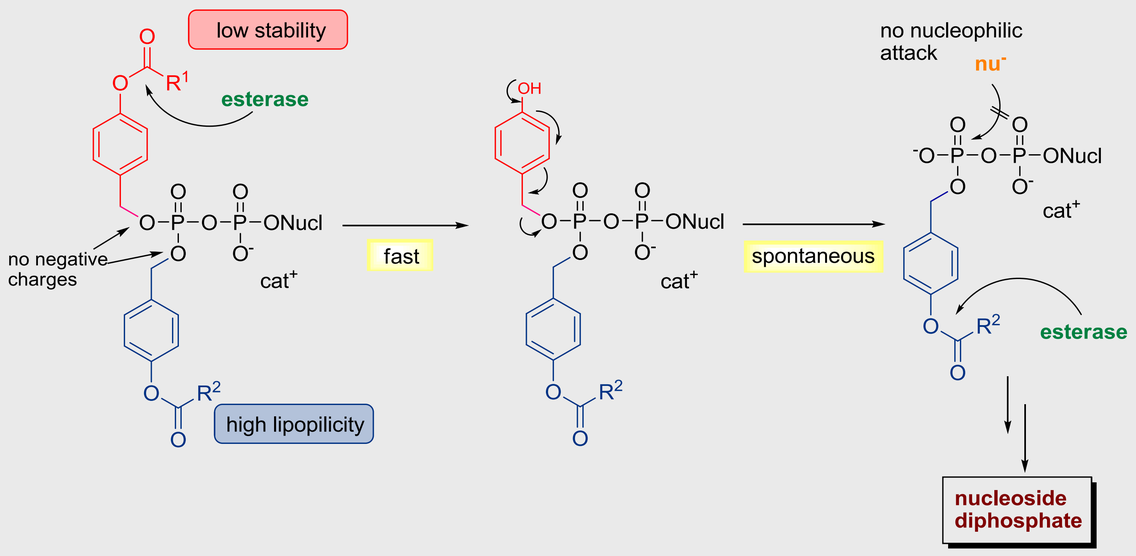


Remarkably, the design of nucleoside diphosphate prodrugs has been addressed very rarely although for instance AZT is only very slowly diphosphorylated to AZTDP by thymidylate kinase (TMP-K).[3] Therefore, we turned our interest to the bioreversible protection of nucleoside diphosphates (NDPs).

Objectives

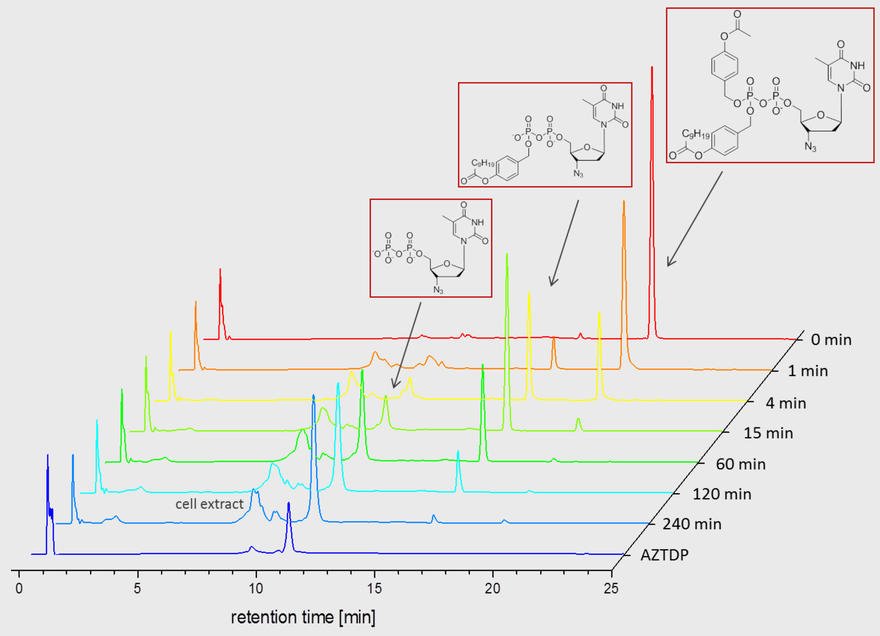
We present a diphosphate prodrug concept using two different bis(acyloxybenzyl)-moieties to neutralize two negative charges at the β-phosphate. Since this concept is dependent on enzymatic hydrolysis, the diphosphate is released preferentially inside cells. The chemical stability at physiological pH should be high. Former studies introduced this concept using Di*PP*ro-NDPs bearing two identical masking groups at the β-phosphate group. However, beside the release of NDP also the monophosphate (NMP) was formed, as a result of a nucleophilic attack at the phosphate anhydride bond. The combination of two different bis(acyloxybenzyl)-moieties – one with low stability and one with high lipophilicity – ensure the fast hydrolysis to an intermediate with high resistance to the pyrophosphate cleavage. The lipophilicity of the other mask still enables the penetration of the cell membrane. We synthesized and studied a series of asymmetric Di*PP*ro-nucleotides with different combination of aliphatic and aromatic moieties at the acyl group.

General Concept



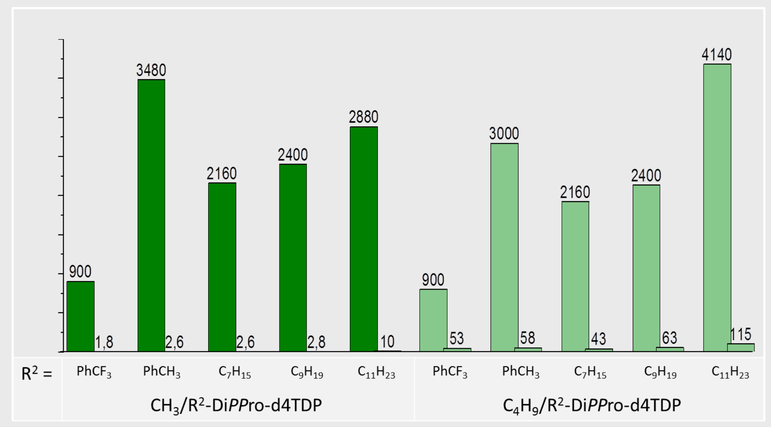
Hydrolysis Studies of Asymmetric Di*PP*ro-Nucleotides

Incubation of Di*PP*ro-nucleotides led to the selective release of nucleoside diphosphate in CEM/0 extract while no formation of the nucleoside monophosphate was observed.



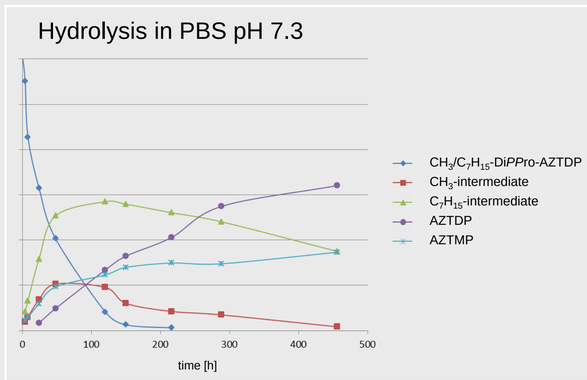
Stability of Di*PP*ro-Nucleotides

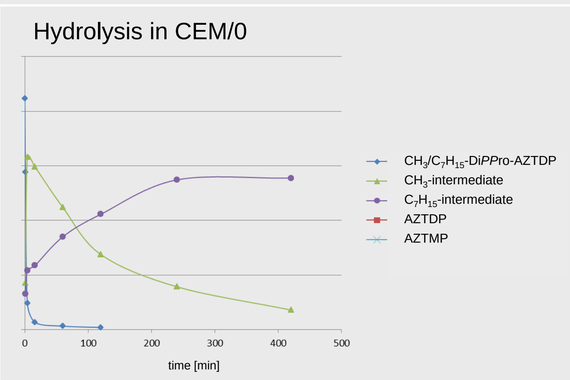
In contrast to the low stability in cell extracts Di*PP*ro-nucleotides are reasonably stable against chemical hydrolysis (phosphate buffer, pH 7.3). The half-lives in PBS (left column) are up to 1000-times higher than those found in cell extract (right column).



left column: $t_{1/2}$ in PBS [min], right column: $t_{1/2}$ in CEM/0 [min],

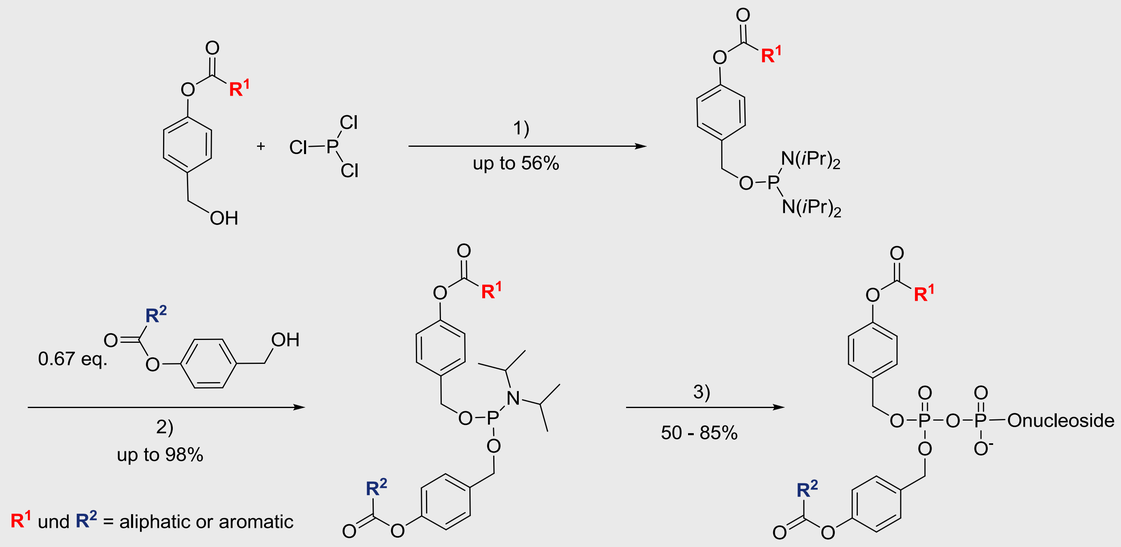
Hydrolysis of CH_3/C_7H_{15}-Di*PP*ro-AZTDP in PBS (pH 7.3) and CEM/0 cell extract





- both intermediates are observed
- slow hydrolysis: concomitant cleavage of the pyrophosphate group → formation of small amounts of NMPs
- only the intermediate with the more lipophilic ester function is formed
- fast hydrolysis: selective release of NDP; no formation of nucleoside monophosphate

Synthesis of Asymmetric Di*PP*ro-Nucleotides



1a) 1 eq. pyridine, - 78 °C to rt, 24 h, THF 1b) 6.1 eq. *N,N*-di*iso*propylamine, -10 °C to rt, 24 h - 48 h, THF; 2) 0.67 eq. 4,5-dicyanoimidazole, 4 °C to rt, 30 - 60 min, CH_3CN; 3a) nucleoside monophosphate, 4,5-dicyanoimidazole, rt, 30 min, CH_3CN 3b) *tert*BuOOH, rt, 15 min, CH_3CN 3c) automatic flash chromatography on rp18, ion exchange, automatic flash chromatography on rp18.

Antiviral Activity and Cellular Cytotoxicity

			EC_{50}[a] [μM]			CC_{50}[b] [μM]
			MT-4/0		MT-4/TK-	MT-4
compound	R^1	R	HIV-1(NL4.3)	HIV-2(ROD)	HIV-2	
CH_3/$PhCF_3$-Di*PP*ro-d4TDP	CH_3	$PhCF_3$	0.4	0.70	i.p.*	112
CH_3/$C_{11}H_{23}$-Di*PP*ro-d4TDP		$C_{11}H_{23}$	< 0.08	< 0.08	i.p.*	19
C_4H_9/$PhCF_3$-Di*PP*ro-d4TDP	C_4H_9	$PhCF_3$	0.18	0.30	i.p.*	35
C_4H_9/$C_{11}H_{23}$-Di*PP*ro-d4TDP		$C_{11}H_{23}$	< 0.08	< 0.08	i.p.*	24
d4T			0.50	0.83	175	176

[a] Antiviral activity in T-lymphocytes: 50% effective concentration; [b] Cytotoxic activity: 50% cytotoxic concentration to reduce cell viability. *in progress

Conclusion

- ✓ Synthesis of asymmetric Di*PP*ro-d4TDPs and -AZTDPs in high purities and good chemical yields
- ✓ Selective and exclusive release of d4TDP or AZTDP in CEM/0 cell extracts
- ✓ High stability against chemical hydrolysis
- ✓ Very promising antiviral data

Di*PP*ro-compounds serve as efficient delivery systems for the intracellular release of nucleoside diphosphates and these compounds can act as prodrugs for NRTI diphosphates

References

1) J. Balzarini, P. Herdewijn, E. De Clercq, *J. Biol. Chem.* **1989**, *264*, 6127-6133; J. Balzarini, *Pharm. World Sci.* **1993**, *16*, 113-126; 2) S.J. Hecker, M.D. Erion, *J. Med. Chem.* **2008**, *51*, 2328-2345; 3) A. Lavie, I. Schlichting, I.R. Vetter, M. Konrad, J. Reinstein, R.S. Goody, *Nature Med.* **1997**, *3*, 922-924; 4) a. H.J. Jessen, T. Schulz, J. Balzarini, C. Meier, *Angew. Chem. Int. Ed.* **2008**, *47*, 8719-8722; T. Schulz, J. Balzarini, C. Meier, *ChemMedChem.* **2014**, *9*, 762-775.