THE Tri*PPP*ro-APPROACH: DEVELOPMENT OF NUCLEOSIDE TRIPHOSPHATE PRODRUGS

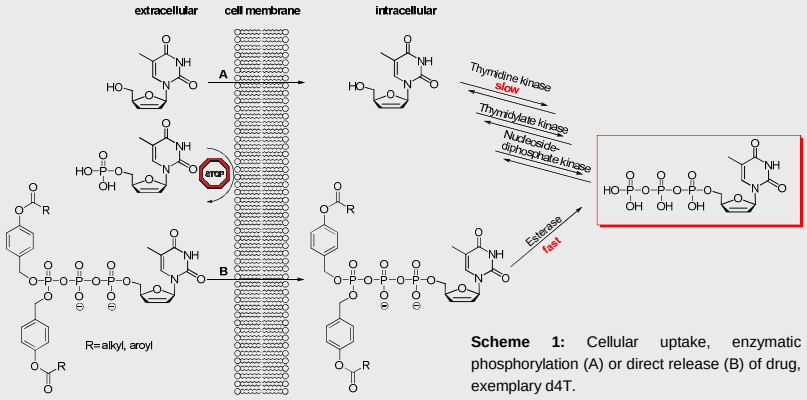
Tristan Gollnest[1], Jan Balzarini[2] and Chris Meier[1*]

[1]*Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany.*
[2]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven (Belgium)*
** chris.meier@chemie.uni-hamburg.de.*

Introduction

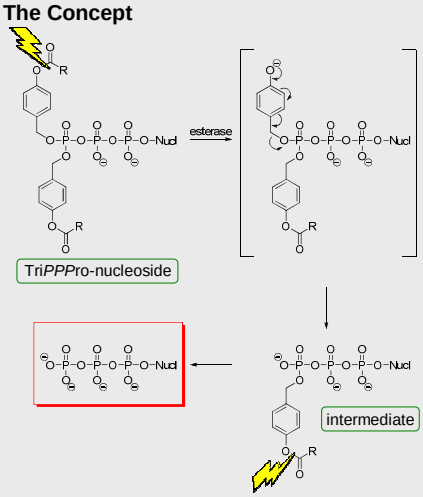
Over the last decades a variety of nucleosides are applied in antitumor and antiviral therapy and play currently an important role in treatment of HIV. Many antiviral drugs are focused on the inhibition of viral reverse transcriptase (RT), which is the key enzyme in the replicative cycle of a virus. However, for example 2',3'-dideoxy- or 3'-modified-nucleosides are limited in their efficiency due to the necessity of intracellular phosphorylation steps by kinases. If the biotransformation into the corresponding NTP occurs insufficiently, the antiviral efficacy is very low. Due to their polarity the application of negatively charged phosphorylated nucleosides is not possible. An option to overcome this problem is the use of lipophilically masked phosphorylated nucleoside analogs, which are able to migrate through the membrane and deliver the corresponding nucleotide by e.g. enzymatic hydrolysis. The *cyclo*Sal-prodrug system was developed for nucleoside monophosphates and has been applied successfully to different nucleoside analogs.[1]

Recently, we reported on a convenient approach called Di*PP*ro-concept for the delivery of nucleoside diphosphates.[2,3] Here, we describe the development of nucleoside triphosphate prodrugs.



Scheme 1: Cellular uptake, enzymatic phosphorylation (A) or direct release (B) of drug, exemplary d4T.

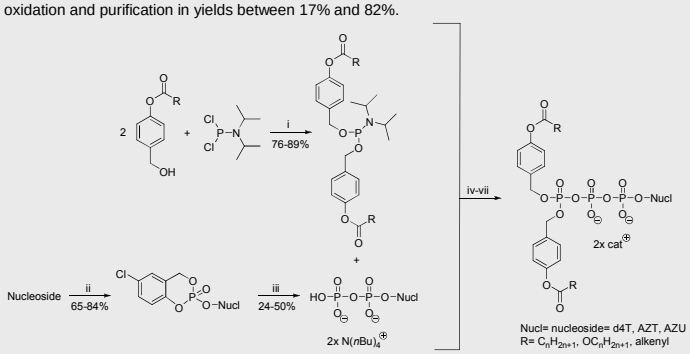
The Concept



Scheme 2: Delivery of NTP by enzymatic cleavage of the masking units.

Preparation of the Tri*PPP*ro-Compounds

*Cyclo*Sal phosphate triesters were used to prepare the nucleoside diphosphates (NDPs).[4] The prodrugs were synthesized by DCI mediated coupling of phosphoramidites with NDP, followed by oxidation and purification in yields between 17% and 82%.



Scheme 3: Reagents and conditions: i) triethylamine, THF, 0 °C-rt, 2 h; ii) 1. 5-chloro-saligenylchlorophosphite, *N,N*-di*iso*propylethylamine, CH_3CN, -20 °C-rt, 3 h, 2. *t*-BuOOH in *n*-decane, 0 °C-rt, 30 min; iii) $(H_2PO_4)Bu_4N$, DMF, rt, 20 h; iv) DCI, CH_3CN, rt, 1 min; v) *t*-BuOOH in *n*-decane, 0 °C, 15 min; vi) Dowex-NH_4^+; vii) RP-18 chromatography

Table 1: Prepared Tri*PPP*ro-nucleosides.

nucleoside	R	Yield [%]
d4T	CH_3	58
	C_2H_5	42
	C_4H_9	47
	C_6H_{13}	43
	C_8H_{17}	31
	C_9H_{19}	27
	$C_{11}H_{23}$	48
	$C_{13}H_{27}$	82
	$C_{15}H_{29}$	66
	$C_{17}H_{35}$	47
	$C_{17}H_{33}$ (8*Z*)	53
	OCH_3	54
	OC_8H_{17}	56
	$OC_{12}H_{25}$	41
AZT	CH_3	17
	C_8H_{17}	28
	$C_{11}H_{23}$	20
AZU	C_8H_{17}	64

Characterization of a d4TTP-Prodrug

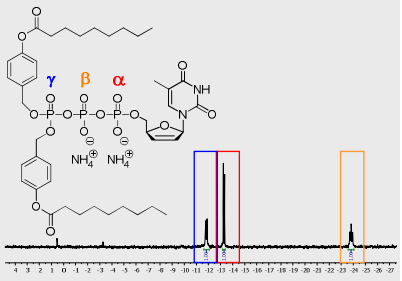


Figure 2: ^{31}P-NMR of Bis-(4-nonanoyloxybenzyl)-d4TTP.

Proofs of d4TTP Formation from the Tri*PPP*ro-Compound

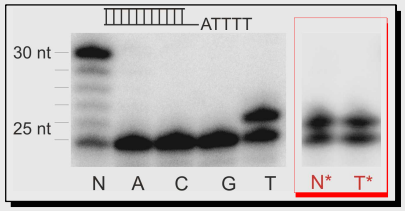


Figure 3: Assay: polymerase β (0.05 units), dNTPs (12.5 µM). Conditions: 15 min, 37 °C.
N*: dATP/dCTP/dGTP+ d4TTP from Tri*PPP*ro-d4TTP (Fig. 4).
T*: only d4TTP from Tri*PPP*ro-d4TTP (Fig. 4).

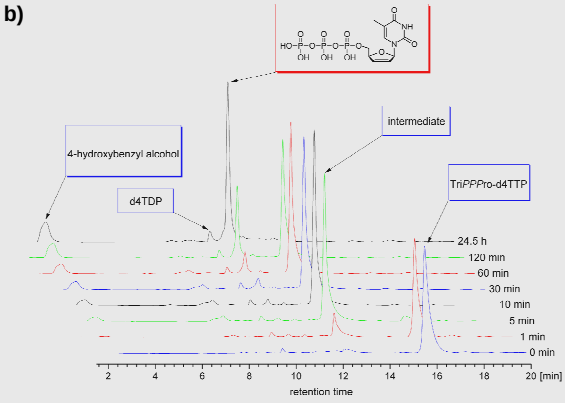


Figure 4: HPLC profile for C-9-Tri*PP*ro-d4TTP after incubation with pig liver esterase (PLE). Fast cleavage of the first mask led to the formation of the intermediate, which selectively delivered the d4TTP.

Anti-HIV-Activity

	EC_{50} (µM)			CC_{50} (µM)
	CEM/0		CEM/TK$^-$	
Compound	HIV-1	HIV-2	HIV-2	CEM/0
R=CH_3	0.10 ± 0.098	0.52 ± 0.16	11 ± 7.5	>250
R=C_8H_{17}	0.035 ± 0.027	0.24 ± 0.049	7.5 ± 3.5	100 ± 2.8
R=$C_{11}H_{23}$	0.044 ± 0.0028	0.50 ± 0.45	3.9 ± 1.1	101 ± 2.1
AZT	0.012 ± 0.0058	0.067 ± 0.018	>250	>250

Table 2: Antiviral data of AZTTP-prodrugs in wild-type CEM cells. Prodrugs showed high activity in thymidine kinases-deficient cells. It increased with the increasing length of the lipophilic chains.

Conclusion

We developed the first example of a nucleoside triphosphate prodrug by masking the γ-phosphate of a NTP with bioreversible groups using a convergent approach in yields up to 82%. A general applicability could allow a variety of drugs entering the cells by passive diffusion and deliver the corresponding biologically active NTP. After delivery, no enzymatic phosphorylation step is needed any longer. Chemical hydrolysis studies in PBS buffer and enzymatic cleavage with pig liver esterase showed the successful selective formation of NTP. In *in vitro* anti-HIV tests, AZTTP-prodrugs were found to be markedly more active than AZT in CEM/TK$^-$ cell assay.

References

[1] C. Meier, *Eur. J. Org. Chem.* **2006**, 1081-1102. **[2]** H. J. Jessen, T. Schulz, J. Balzarini, C. Meier, *Angew. Chem. Int. Ed.* **2008**, *47*, 8719 –8722. **[3]** T. Schulz, J. Balzarini, C. Meier, *ChemMedChem* **2014**, *9*, 762-775. **[4]** S. Warnecke, C. Meier, *J. Org. Chem.* **2009**, *74*, 3024-3030.