Universität Hamburg
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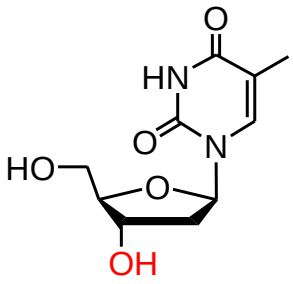
Developing Prodrugs of antivirally active Nucleoside Triphosphates - Against all odds, it works!

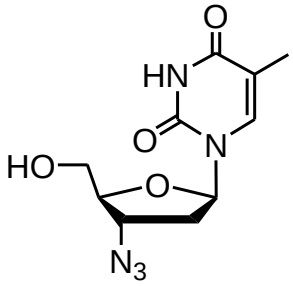
RSC-Meeting, London, England, April 17th, 2015

Prof. Dr. Chris Meier, Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Germany

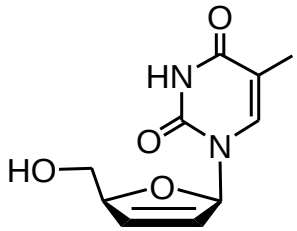
Antivirally active Nucleosides



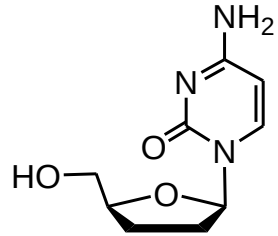
Thymidine



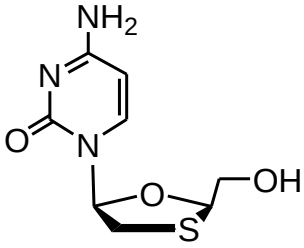
AZT (1987)



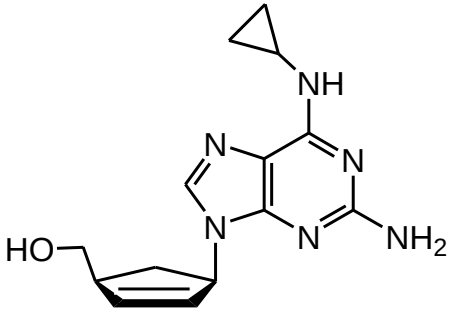
d4T (1994)



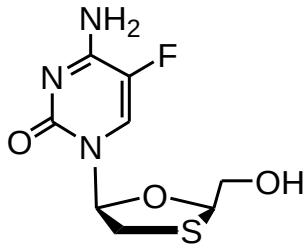
ddC (1992)



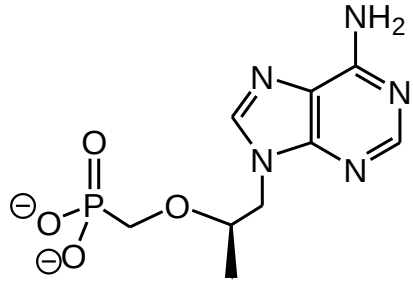
3TC (1995)



Abacavir (1998)



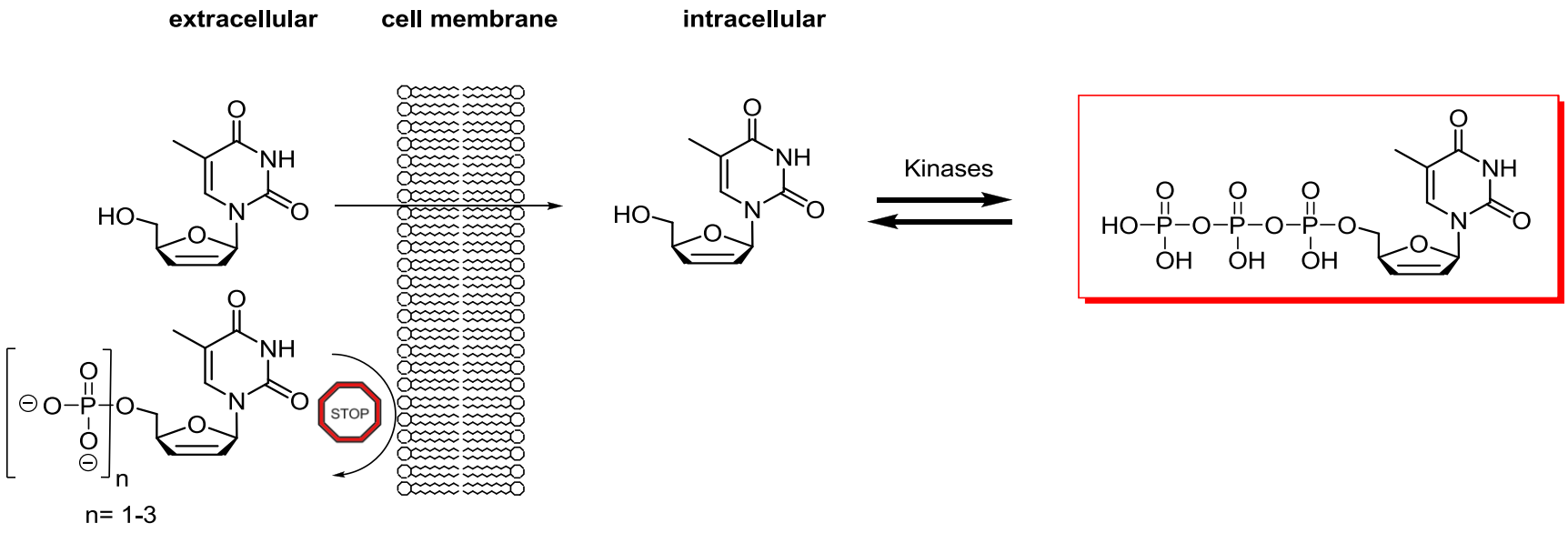
FTC (2006)



PMPA (Tenofovir 2006)

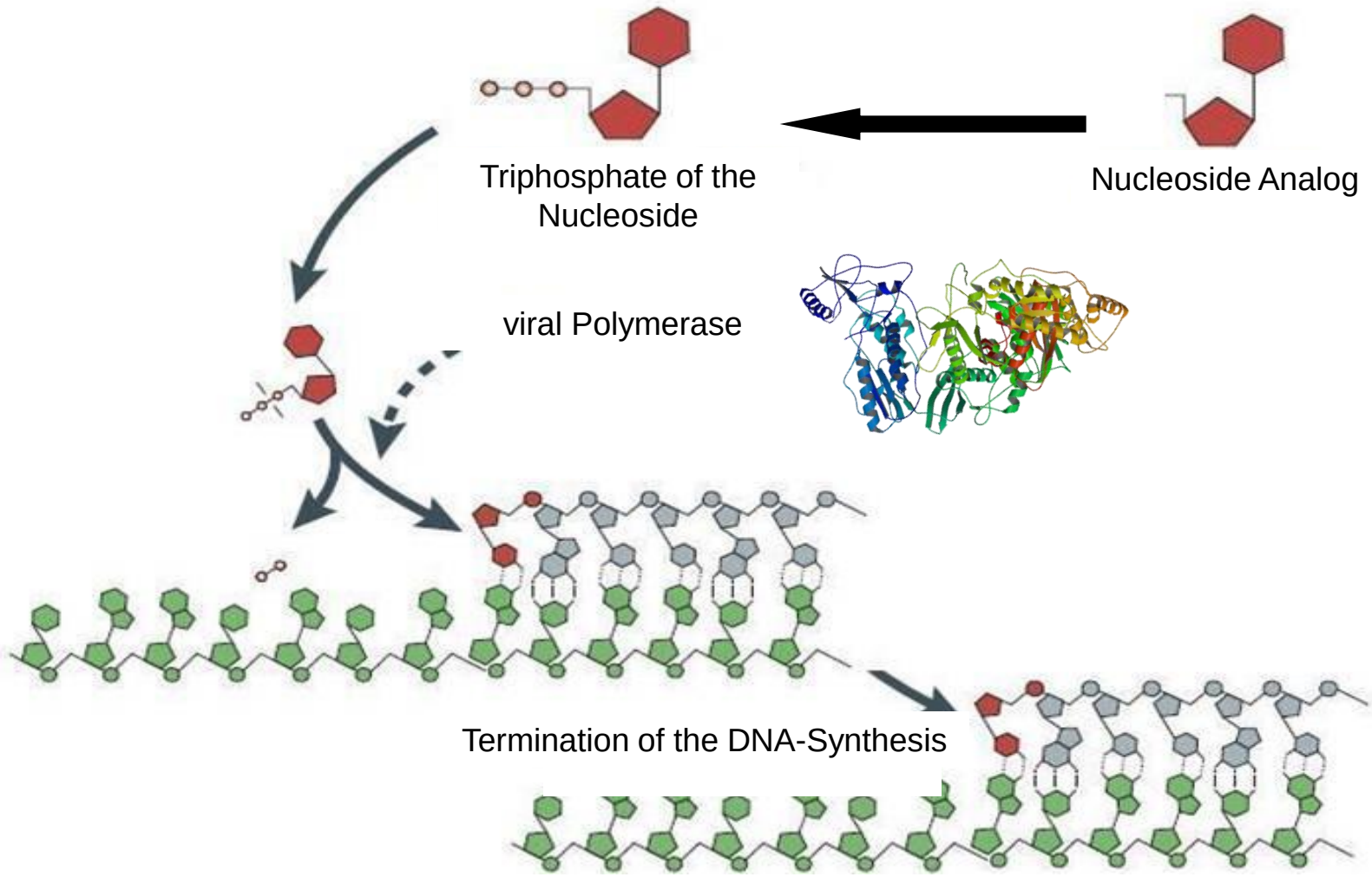
J. L. Marx, AIDS, *Science* **1968**, *231*,1504; S. A. Riddler, R. E. Anderson, J. W. Mellors, *Antiviral Res.* **1995**, *27*, 189-203; S. M. Daluge, M. T. Martin, B. R. Sickles, D. A. Livingston, *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 297; R. S. Schinazi *et. al*, *Antimicrob. Agents Chemother.* **1992**, *36*, 2423

Metabolism of Nucleoside Analogues



- Application of the drug may be limited due to the lack of intracellular phosphorylation!!!
- but: the use of phosphorylated nucleosides is not possible!!!

Mechanism of Action of Nucleoside Drugs

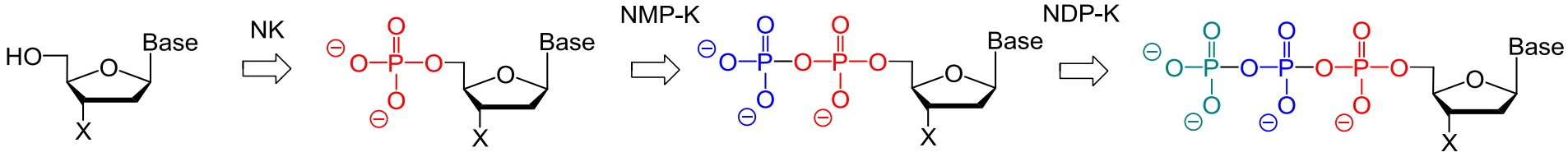


E. De Clercq, *Nat. Rev. Drug Discov.* **2002**, *1*, 13

Nucleotide Prodrugs from the Meier Lab

Synthesis
(novel structure)

Kinase Bypass



NMP

NDP

NTP

*cyclo*Sal-Approach

Di*PP*ro-Approach

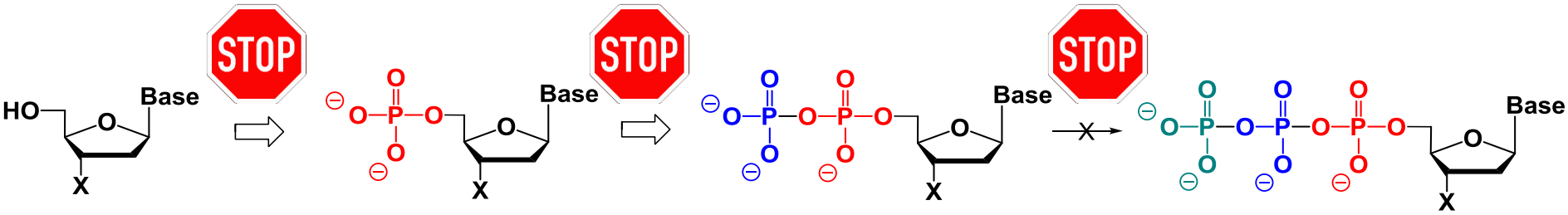
Tri*PPP*ro-Approach

Eur. J. Org. Chem. **2006**, 1081-1102

In preparation

Angew. Chem. Int. Ed. **2008**, *47*, 8719-8722 (“Top 10% papers”)
ChemMedChem, **2014**, *9*, 762-775 („very important paper“)

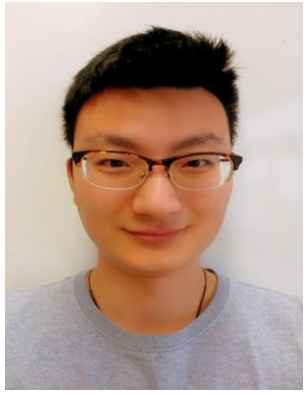
Towards Tri*PPP*ro-Prodrugs



Nucleoside NMP NDP NTP

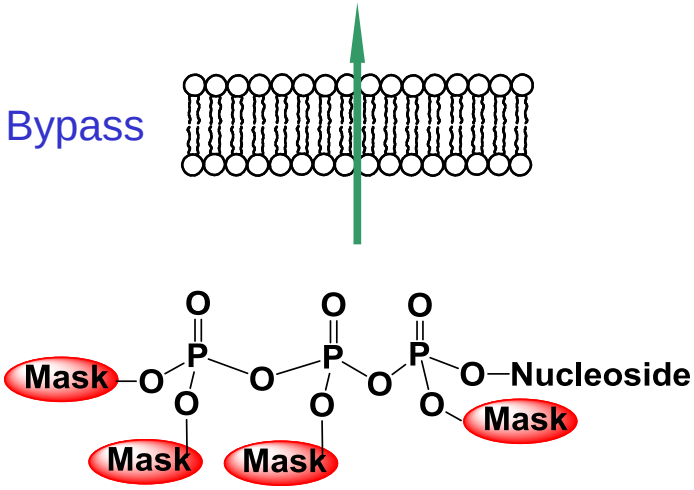
Tristan Gollnest

Tobias Nack



Chenglong Zhao

Kinase Bypass



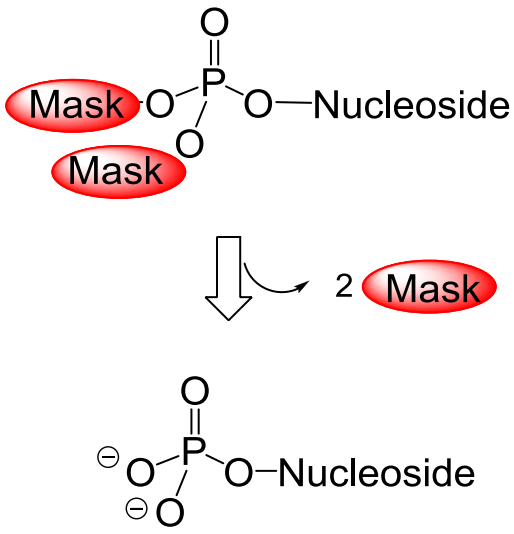
Nucleoside Di- or Triphosphate Prodrugs?

“Direct delivery of triphosphate or diphosphate forms of nucleoside analogs would be desirable but is impractical because of their instability during synthesis.“

X. Tan, K. Chu; *Advanced Drug Delivery Reviews* **1999**, *39*, 117-151

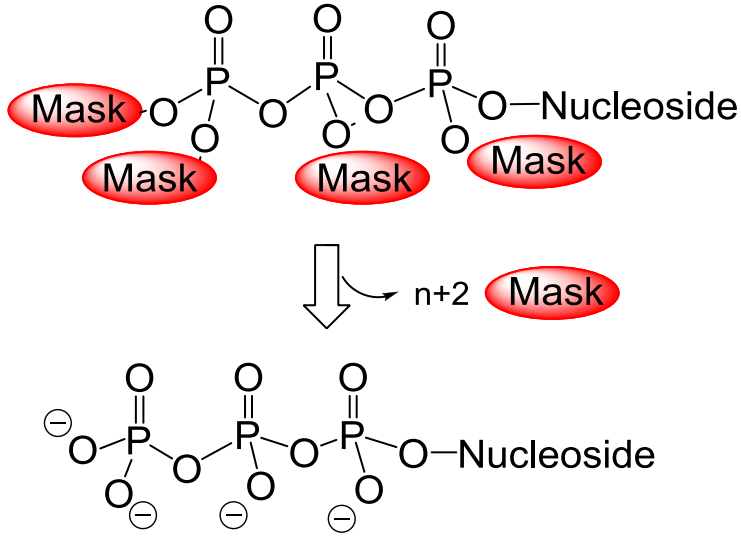
NMP- vs. NTP-Prodrugs

Nucleotide Prodrugs



either by controlled chemical hydrolysis

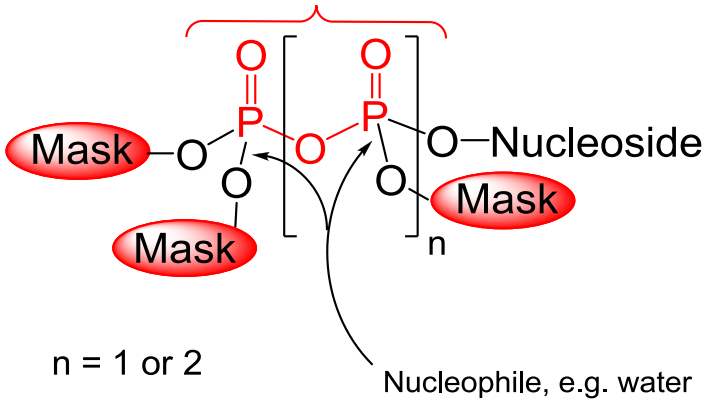
NTP-Prodrugs (fully "masked")



Nucleoside triphosphate

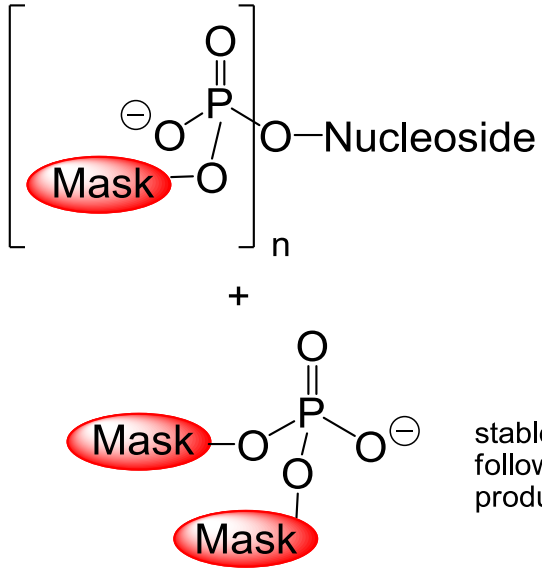
Specific Problem with ND(T)P-Prodrugs

fully masked
Phosphate Anhydride = chemically labile!



either by chemical hydrolysis
or by enzymatic cleavage ?

Pyrophosphate Cleavage

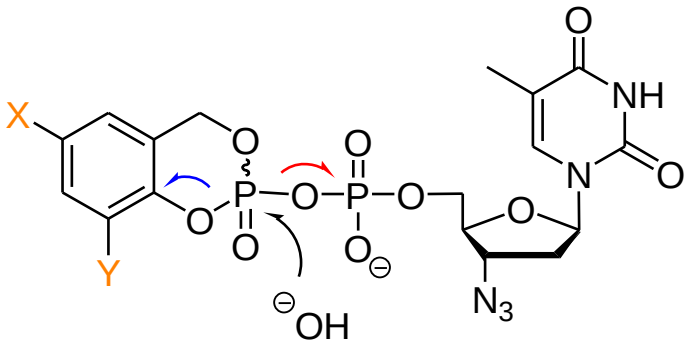


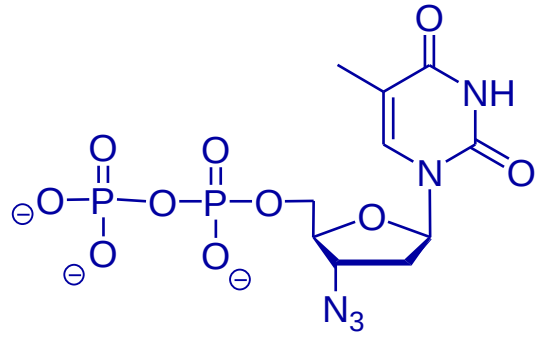
stable or
follow-up
products ?

⇨ To reduce chemical lability, leave a charge at the α- and β-phosphorus atom !

1. No attack at the α- or β-phosphorus atom
2. Mono- or Diphosphate is a bad leaving group

*cyclo*Sal-NDP-Prodrugs

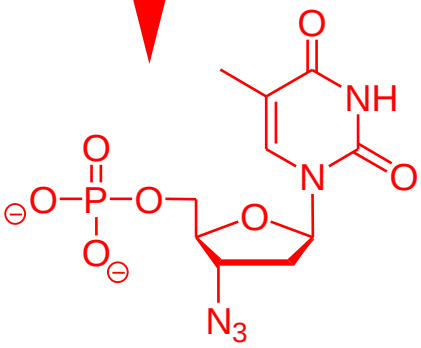




X= H; Y= Me : 1%

X= Cl; Y= H : 25%

AZTDP



AZTMP

X= H; Y= Me : 99%

X= Cl; Y= H : 75%

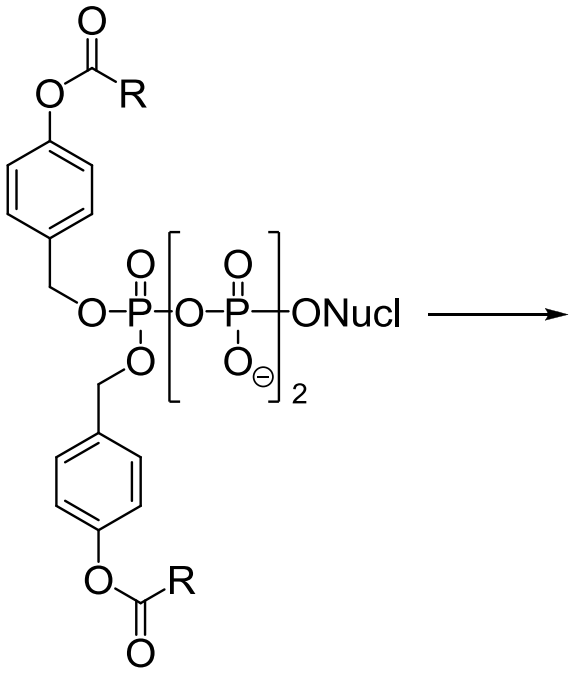
Properties:

- Difficult purification / poor yields
- AZTDP/AZTMP ratio correlates with stability
- Insufficient AZTDP delivery

Consequence: Do not „touch“ the Pyrophosphate !

Enzyme-cleavable Tri*PPP*ro-Compounds

Acceptor

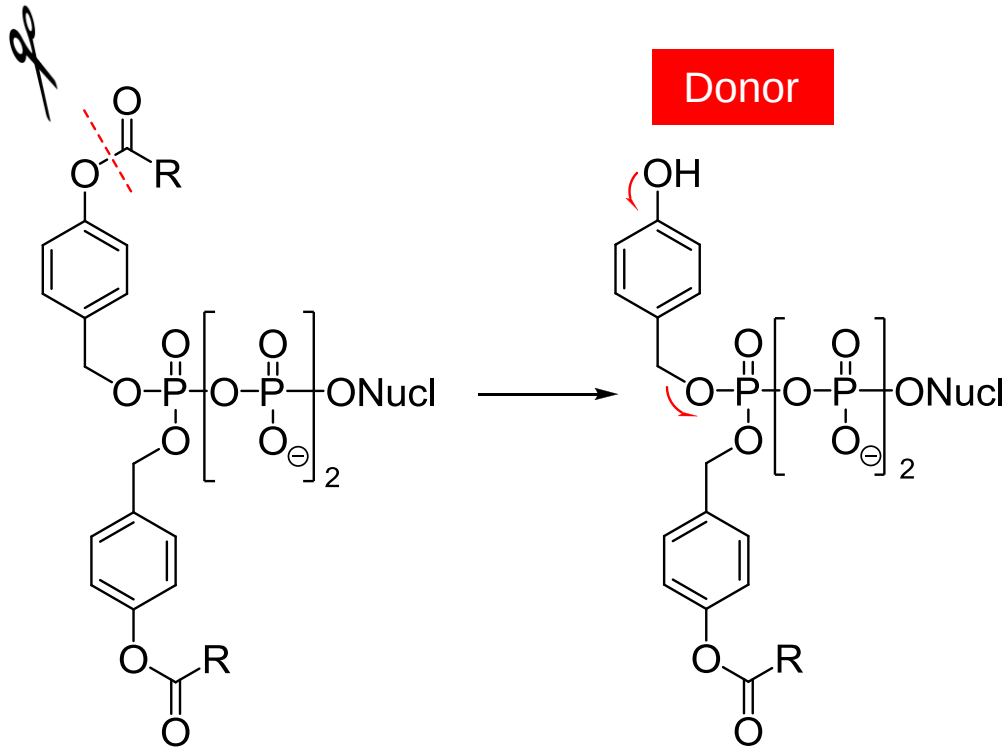


Tri*PPP*ro-NTP

Enzyme-cleavable Tri*PPP*ro-Compounds

Acceptor

Donor

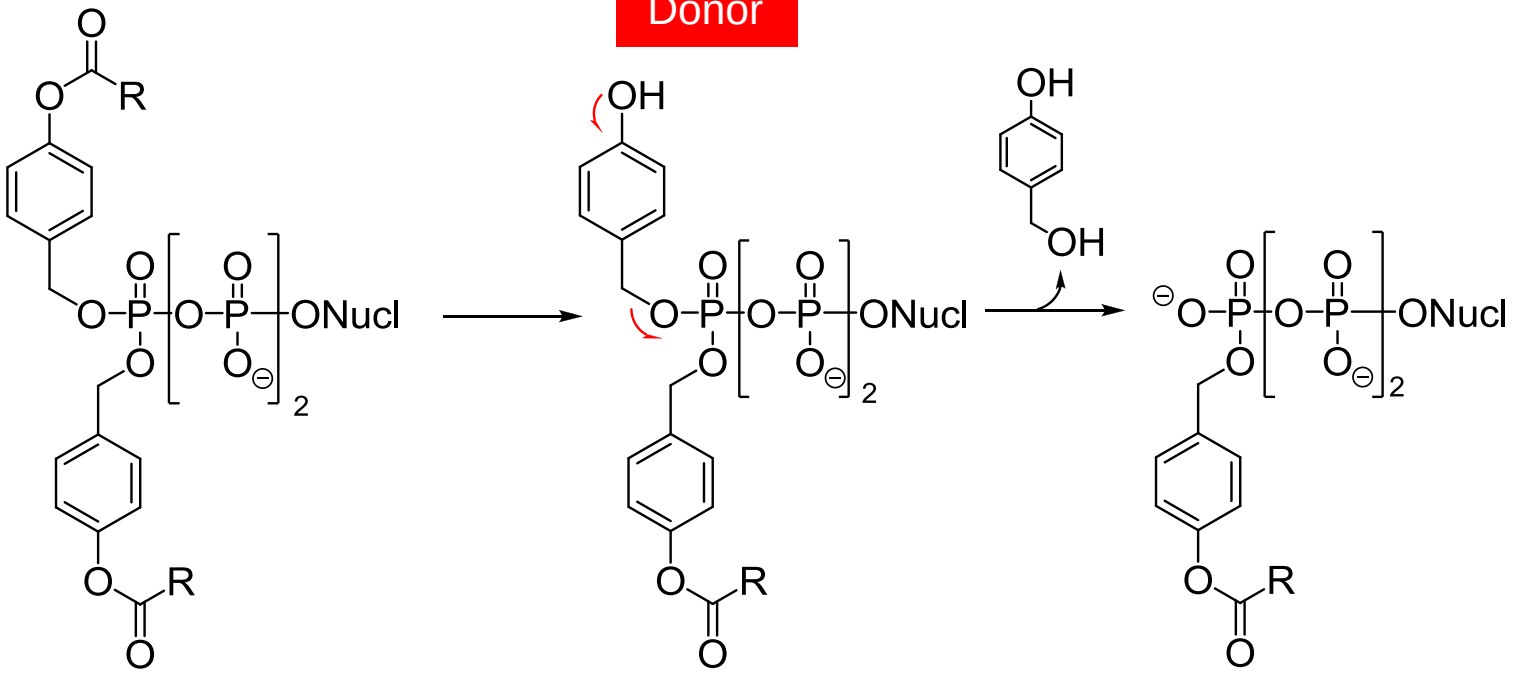


Tri*PPP*ro-NTP

Enzyme-cleavable Tri*PPP*ro-Compounds

Acceptor

Donor

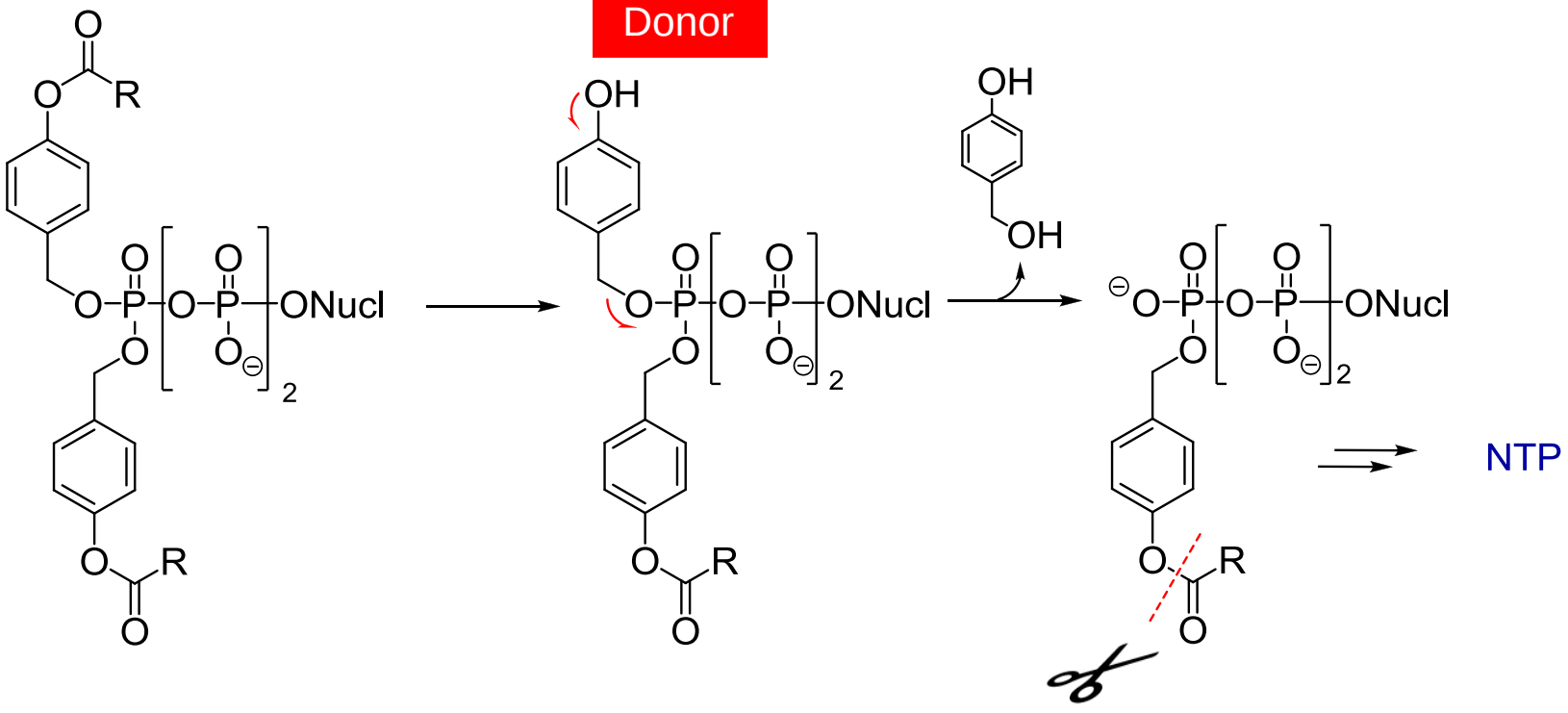


Tri*PPP*ro-NTP

NTP Delivery System: Tri*PPP*ro-Compounds

Acceptor

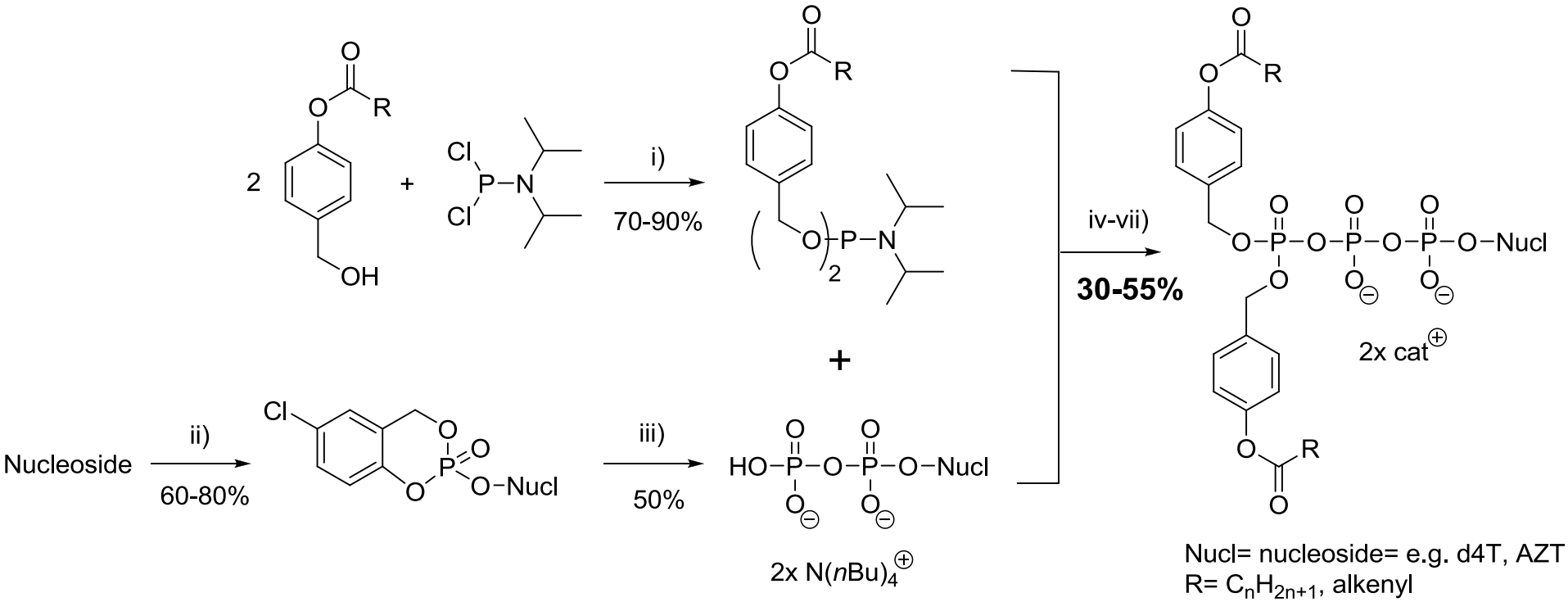
Donor



Features:

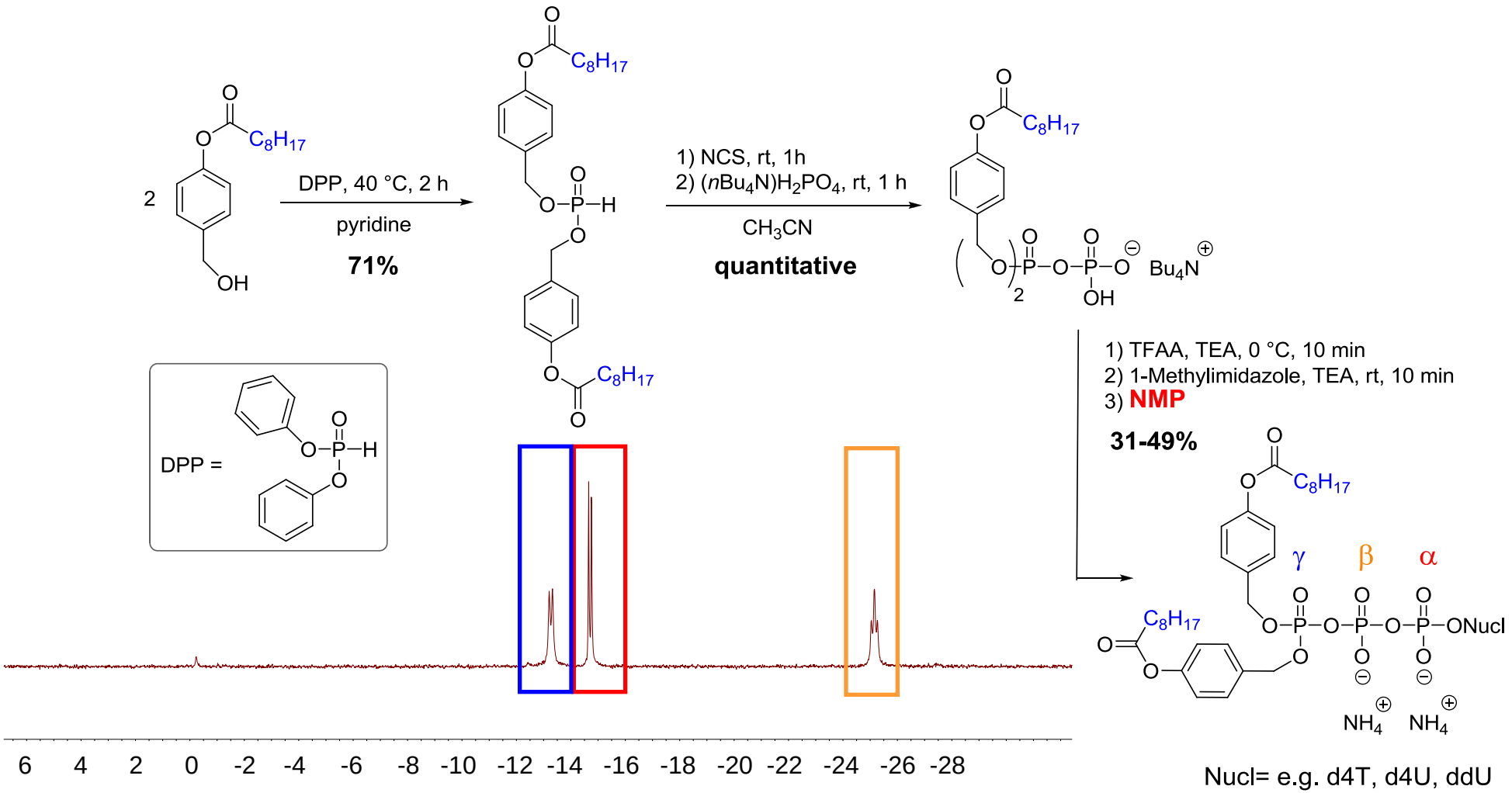
1. charged α and β-phosphorus atom; 2. no activation needed involving the pyrophosphate unit; 3. enzymatic activation; 4. „tunable“ polarity and „tunable“ stability (R)

Phosphoramidite Chemistry



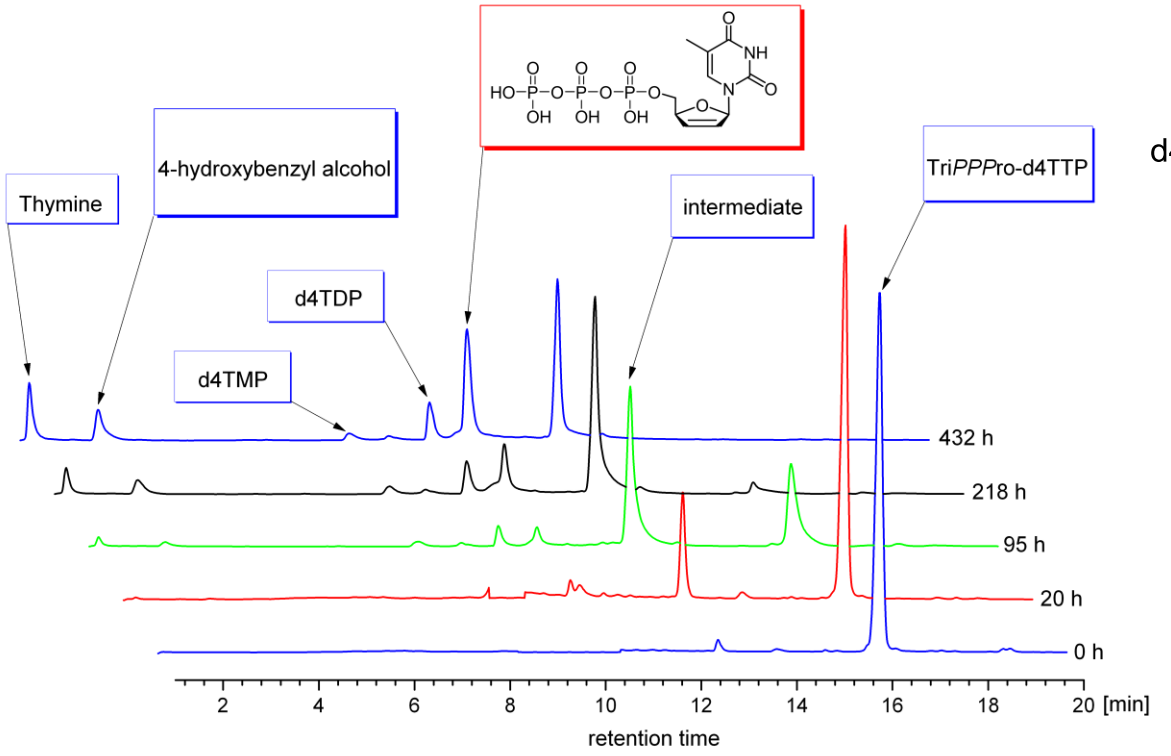
Reagents and conditions: i) triethylamine, THF, 0 °C to rt, 20 h; ii) a. 5-chlorosaligenylchlorophosphite, DIPEA, CH_3CN, -20 °C to rt, 3 h; b. *t*-BuOOH, 0 °C to rt, 30 min; iii) $(H_2PO_4)Bu_4N$, DMF, rt, 20 h; iv) 1 eq. NDP; 1.5 eq. phosphoramidite, 1.7 eq. DCI, CH_3CN, rt, 1 min; v) 1.5 eq. *t*-BuOOH, 0 °C, 20 min; vi) Dowex-NH_4^+; vii) RP-18 chromatography

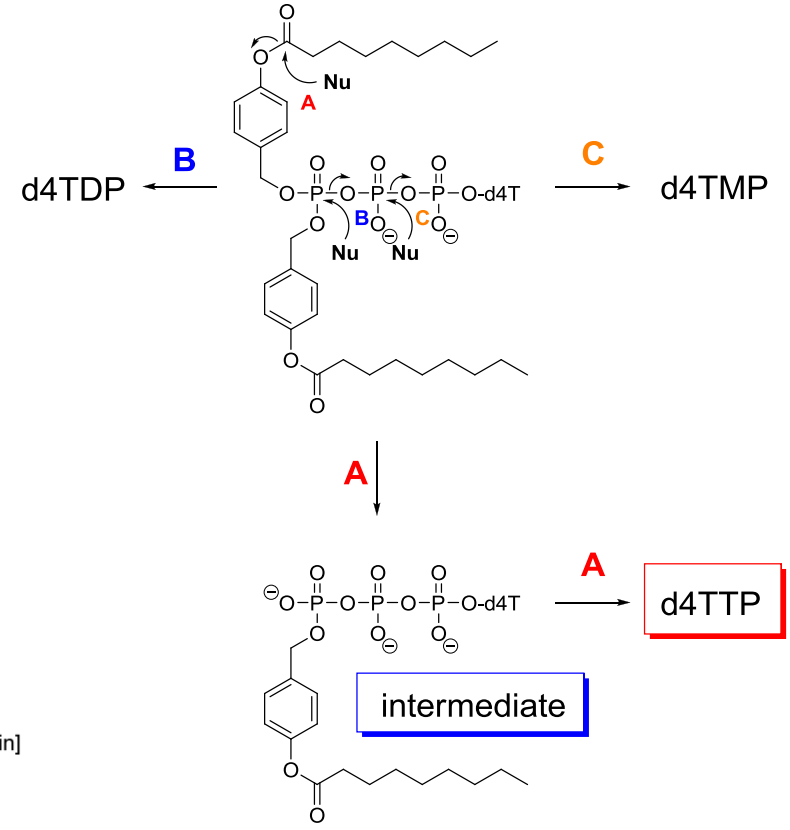
H-Phosphonate Chemistry



Nucl= e.g. d4T, d4U, ddU

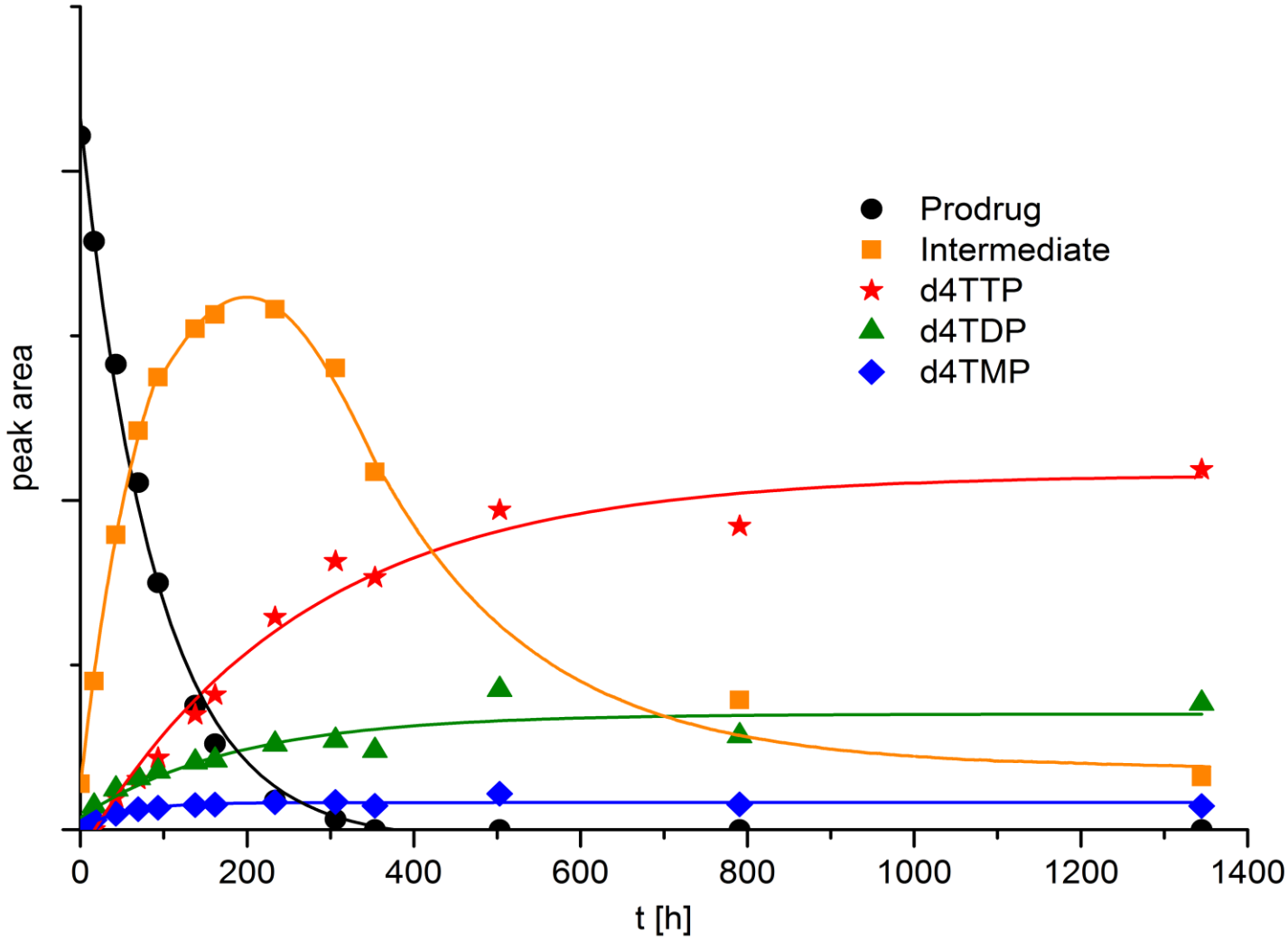
NTP-Formation in aqueous PBS (pH 7.3)



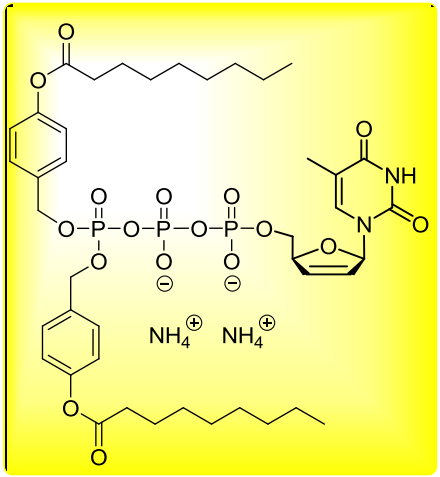


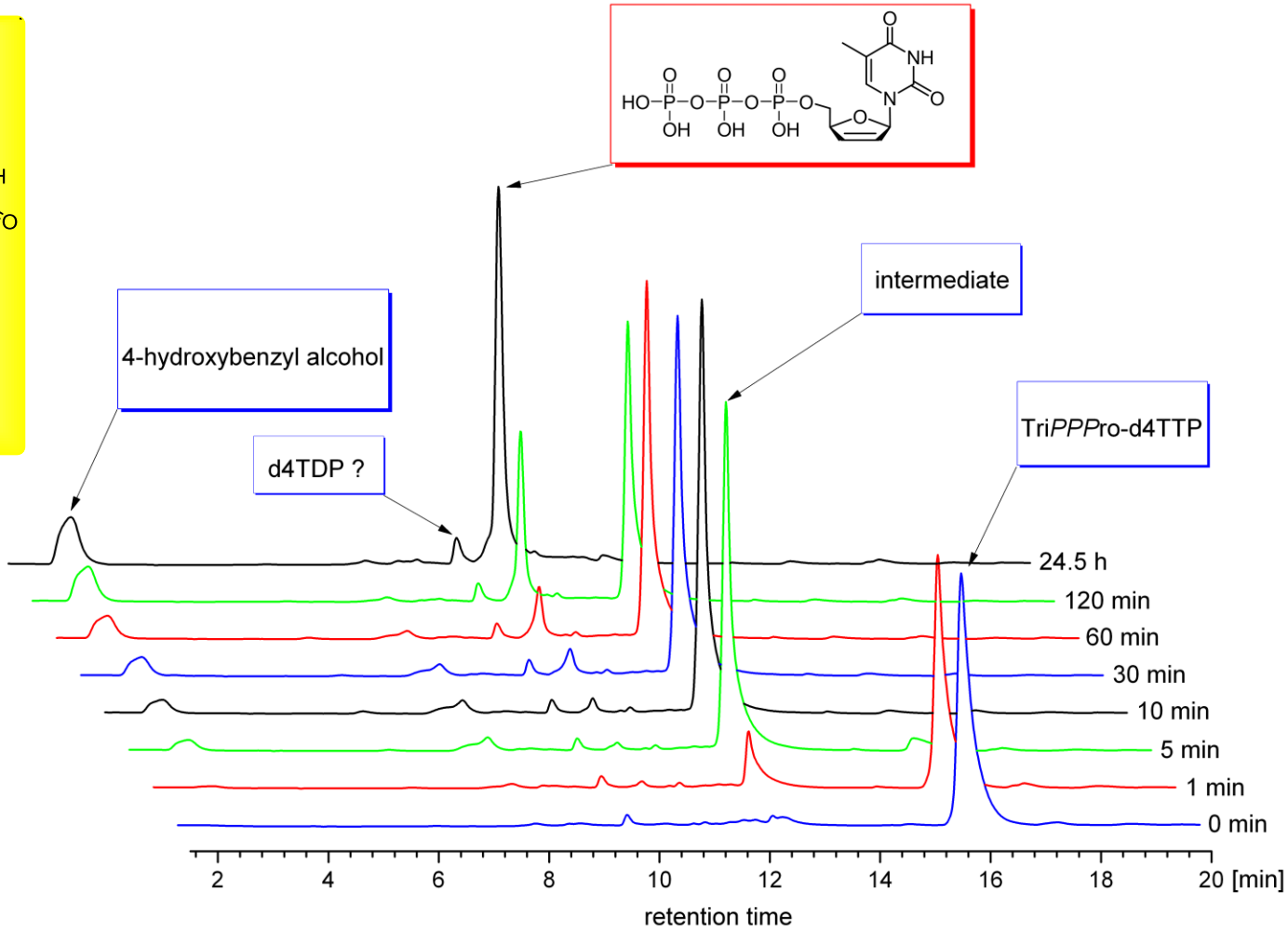
➡ Chemical cleavage in PBS showed the *predominant* formation of NTP !

Chem. Hydrolysis of Tri*PPP*ro-Compounds



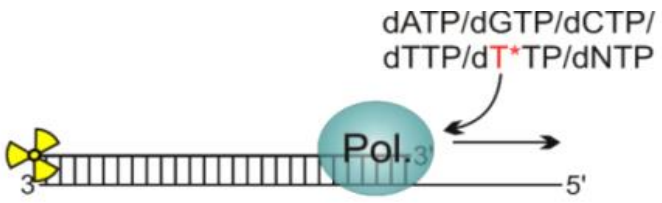
PLE Cleavage of Tri*PPP*ro-d4TTP





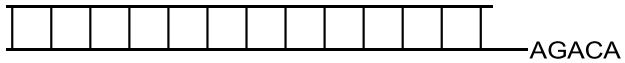
➡ Pig liver esterase (PLE) incubation showed almost selective formation of NTP

Primer Extension Assay of Tri*PPP*ro-dNTPs



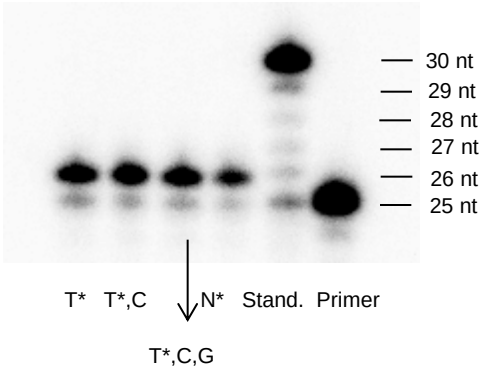
Assay: HIV-RT, dNTP´s [2.5 µM]

Conditions: 37 °C, 12 min.

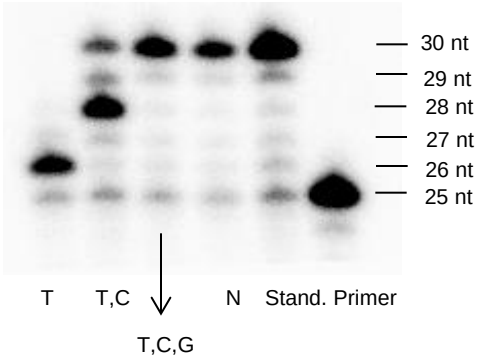


Thiago Dinis de Oliveira

d4T-TP (from the prodrug)



dT-TP (from the prodrug)

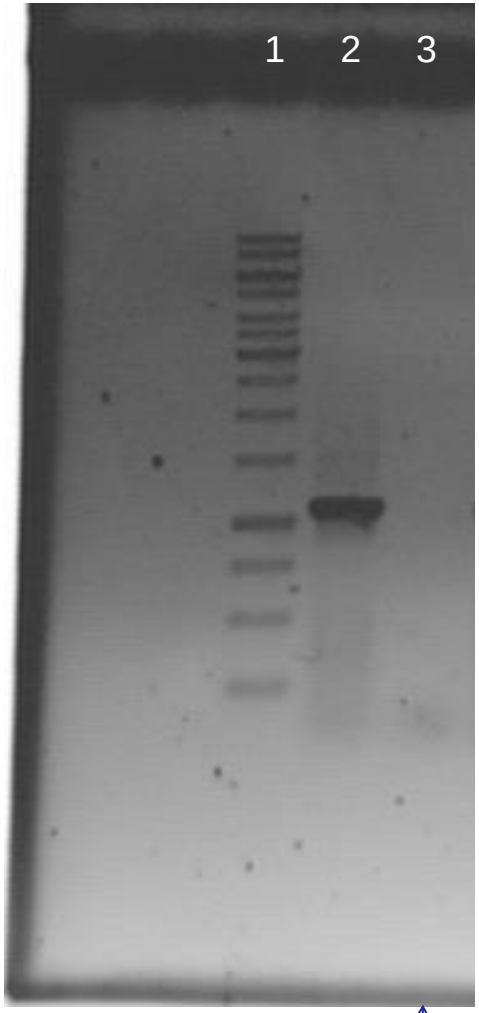


Presented at:

International Conference on Antiviral Research (ICAR), Raleigh, USA, May 2014

International Round Table on Nucleotides and Nucleic Acids (IRT), Poznan, Poland, August 2014

Proof of dNTP Delivery by PCR



no amplification product

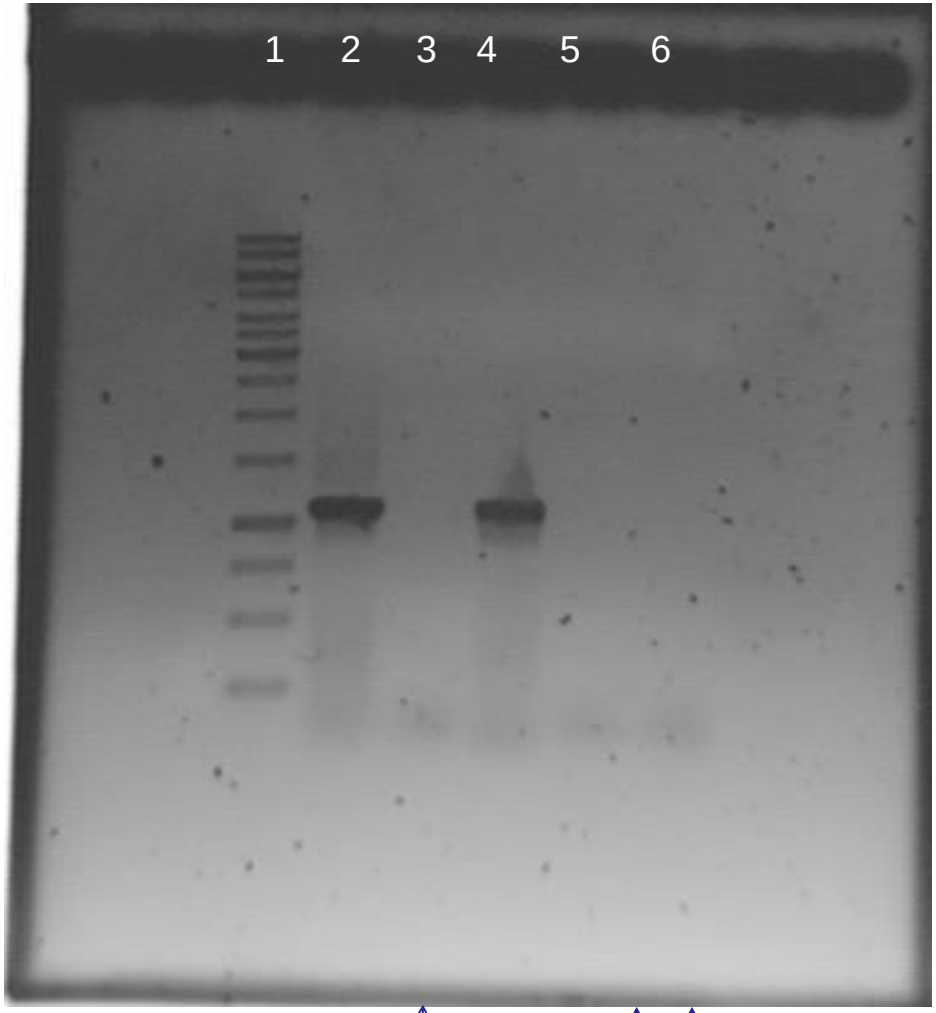
lane 1: standard

lane 2: reference; <u>all</u> dNTPs present

lane 3: dATP, dCTP, dGTP

Enzyme: Fire Pol-polymerase

Proof of dNTP Delivery by PCR



lane 1: standard

lane 2: reference; <u>all</u> dNTPs present

lane 3: dATP, dCTP, dGTP

lane 4: dATP, dCTP, dGTP + **dTTP-prodrug**

lane 5: dATP, dCTP, dGTP + **d4TTP-prodrug**

lane 6: dATP, dCTP, dGTP + **carba-dTTP-prodrug**

25 mM solution of the dNTP-prodrugs;
Enzyme: Fire Pol-polymerase

→ hydrolysis and delivery of dTTP or T-analogue triphosphate by esterase

→ **successful amplification or chain termination !**

Anti-HIV Activity of d4TTP-Prodrugs

Compound	EC_{50} (µM)			CC_{50} (µM)
	CEM/0		CEM/TK$^-$	
	HIV-1	HIV-2	HIV-2	CEM/0
R=CH_3	0.43 ± 0.25	0.72 ± 0.16	>10	63 ± 2
R=C_4H_9	0.40 ± 0.00	1.05 ± 0.30	>10	58 ± 3
R=C_8H_{17}	0.31 ± 0.01	0.62 ± 0.3	2.26 ± 1.03	52 ± 1
R=$C_{11}H_{23}$	0.21 ± 0.01	0.27 ± 0.06	**0.72 ± 0.16**	26 ± 0
R=$C_{17}H_{35}$	0.17 ± 0.00	0.31 ± 0.00	**0.28 ± 0.04**	29 ± 9
d4T	0.84 ± 0.08	0.75 ± 0.49	132 ± 4.8	> 250

➡ d4TTP-prodrugs showed very good antiviral activity in CEM cells in dependence of the length of the alkyl chain

collaboration with J. Balzarini, D. Schols, Rega-Institute, Leuven

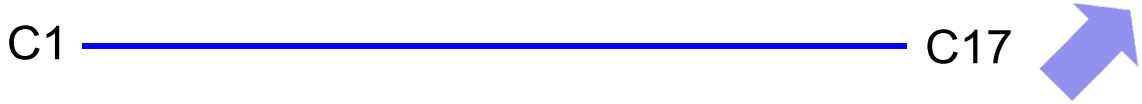
Symmetric Tri*PPP*ro-Nucleotides

Summary of the results (dependence of the alkyl chain length)

Stability $t_{1/2}$ *

Lipophilicity

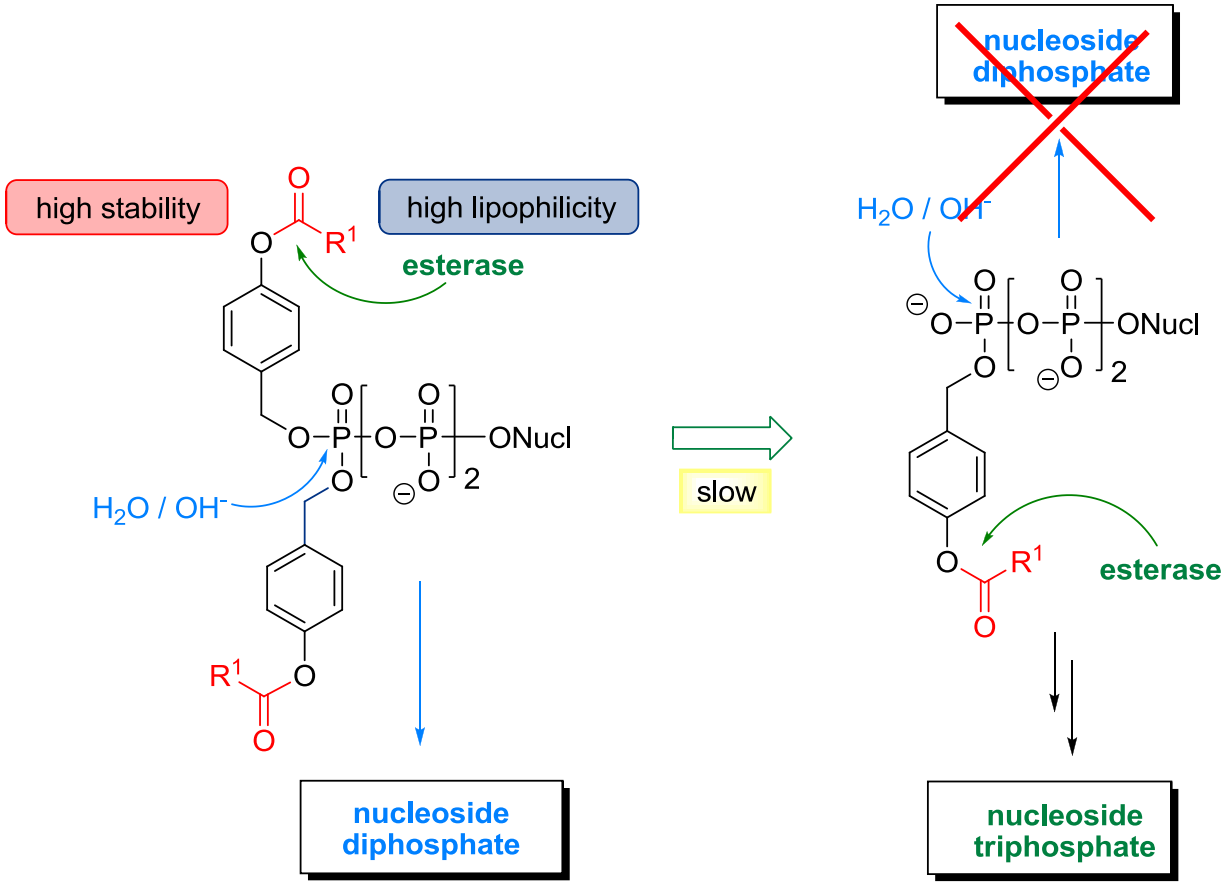
Antiviral Activity



Problem:

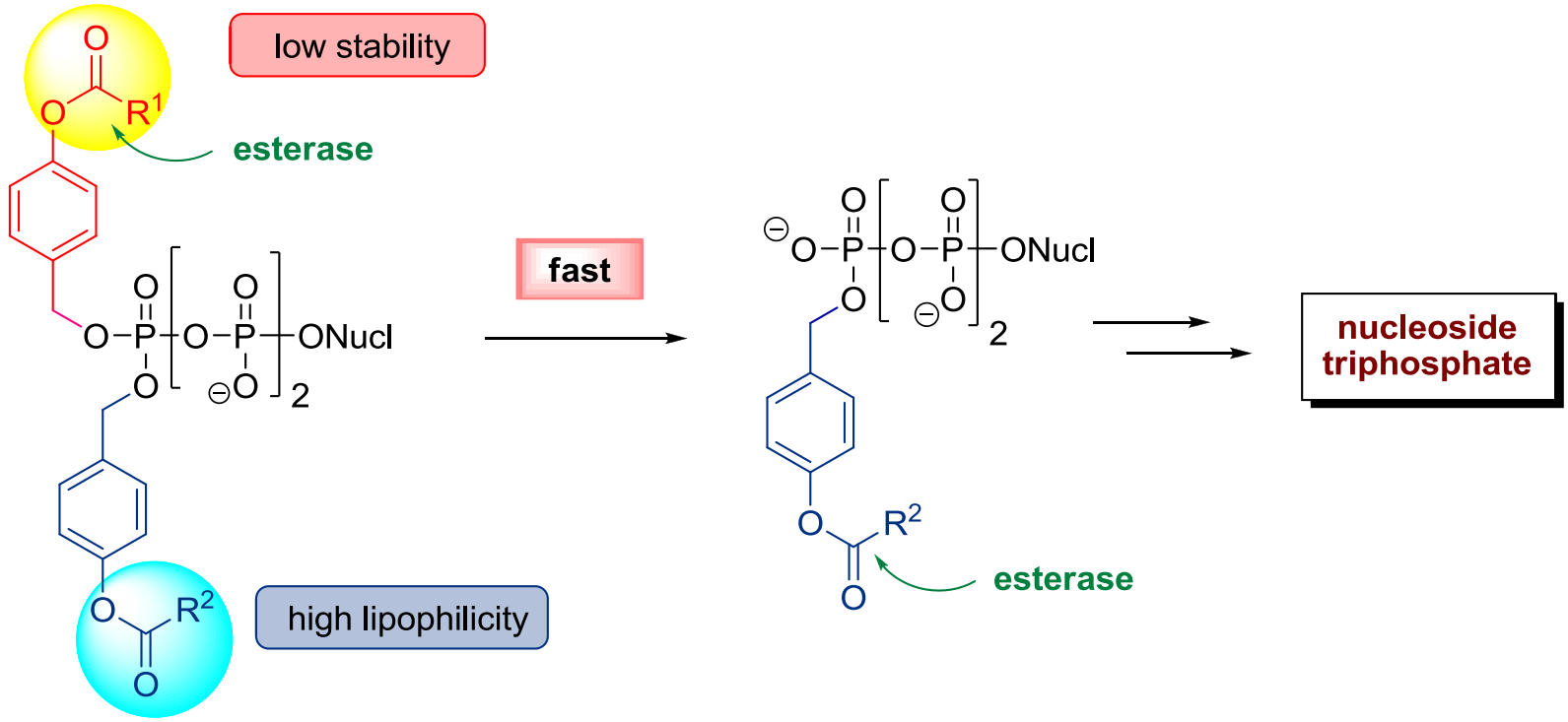
Formation of NMP and NDP *

Symmetric Tri*PPP*ro-Nucleotides



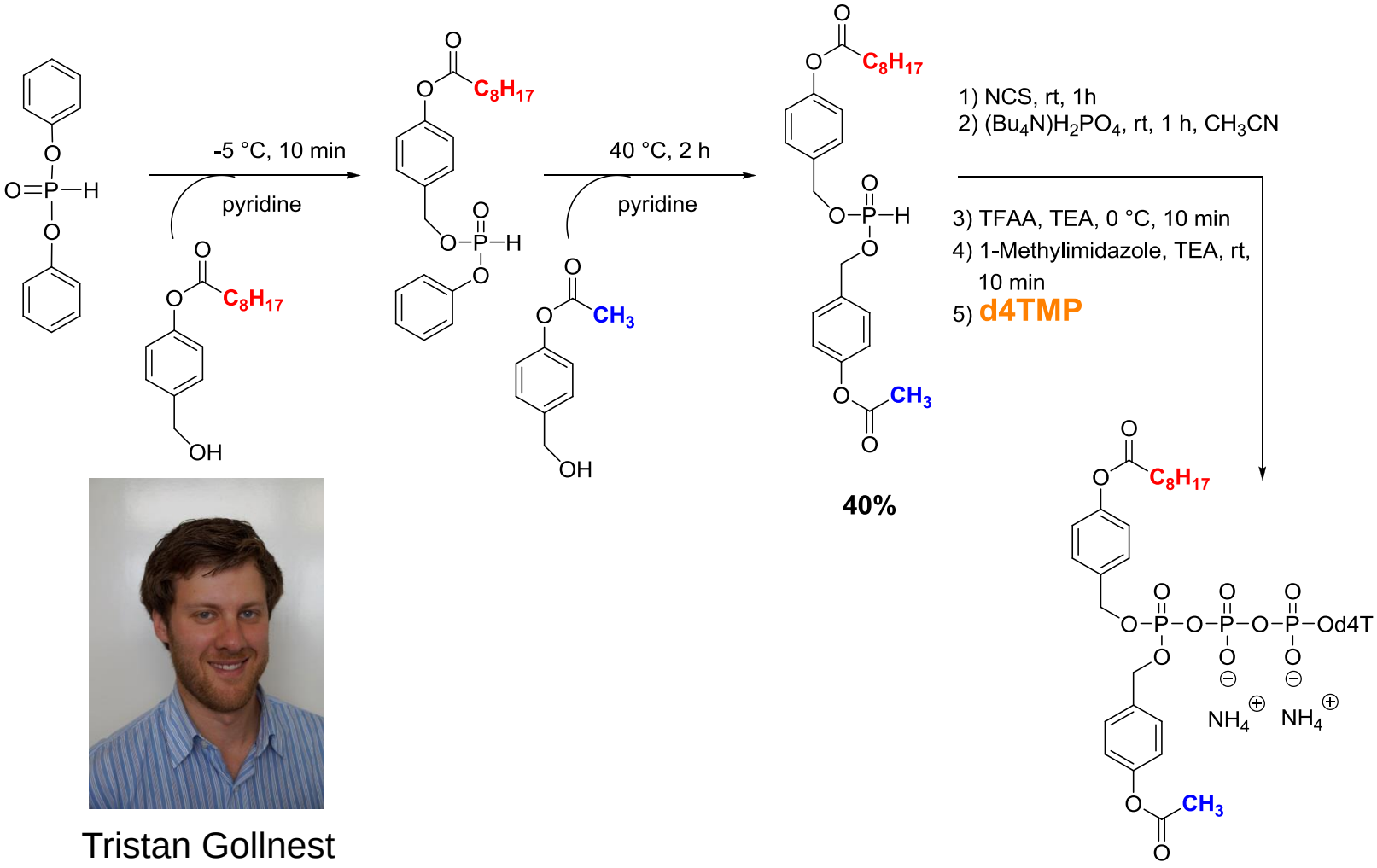
Non-symmetric Tri*PPP*ro-Nucleotides

modified concept:



Patent filed: DE 102014112055.8; date: 22.08.2014

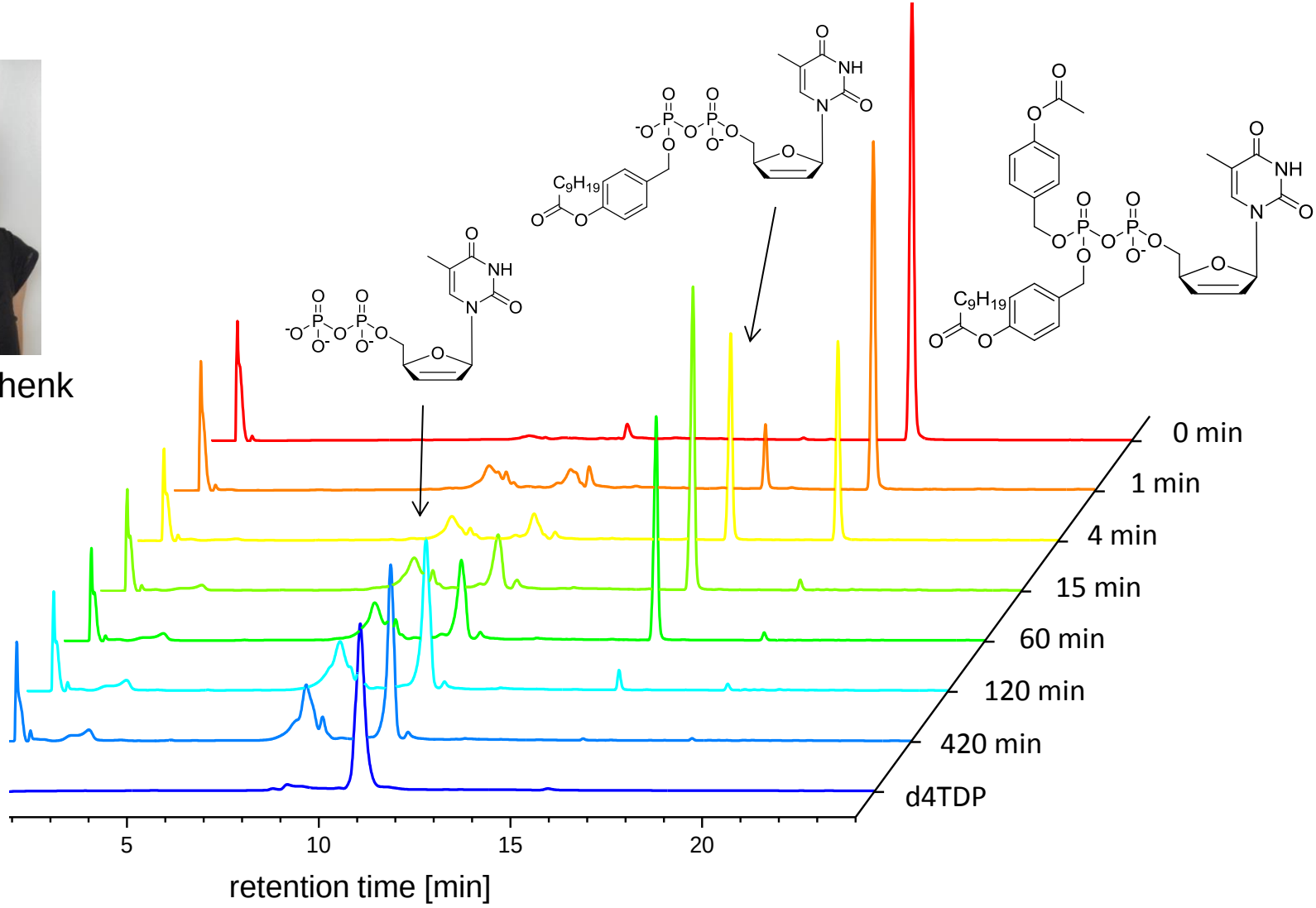
Synthesis of non-symmetric Tri*PPP*ro‘s



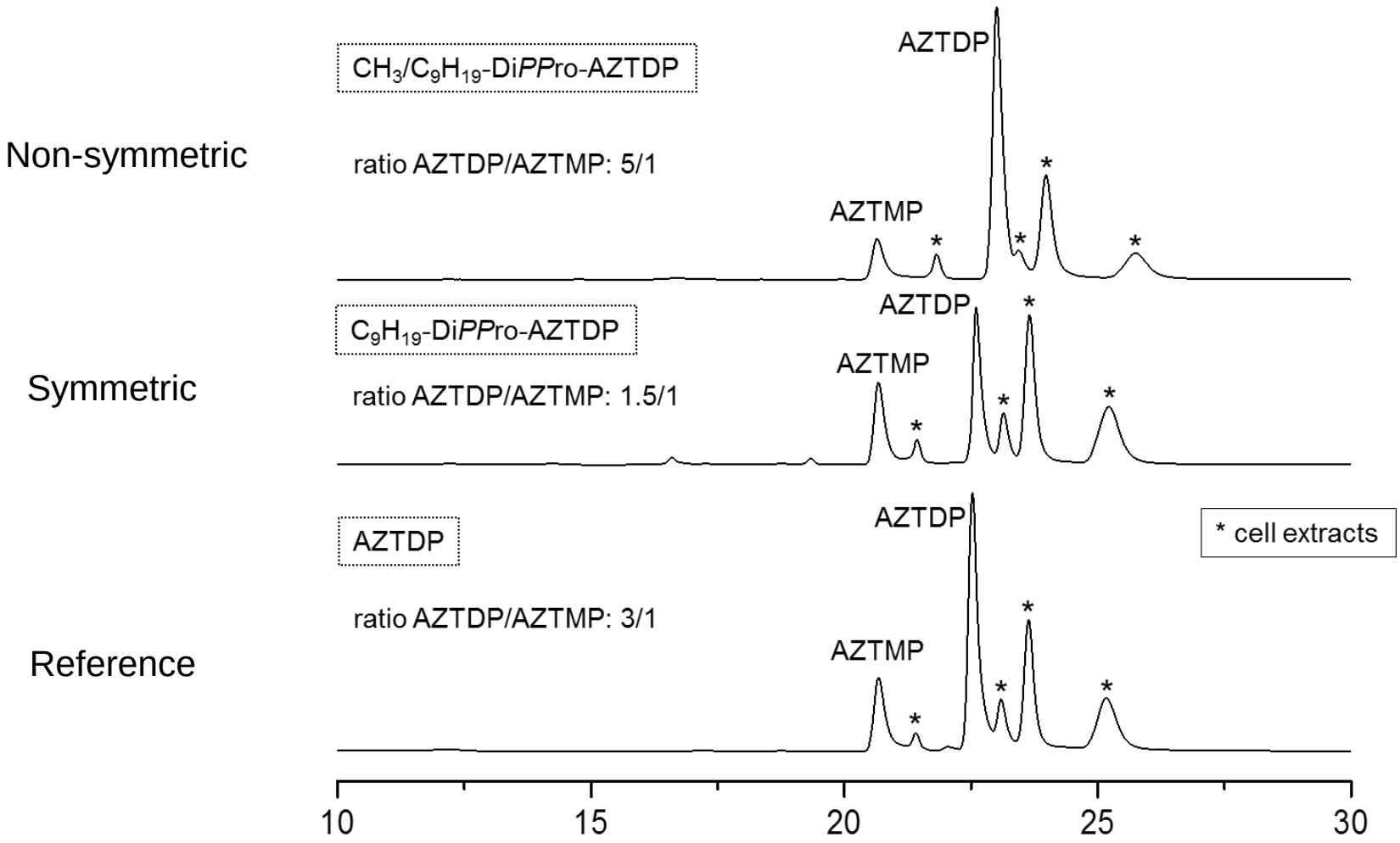
Tristan Gollnest

Selective d4TDP Formation in Cell Extracts

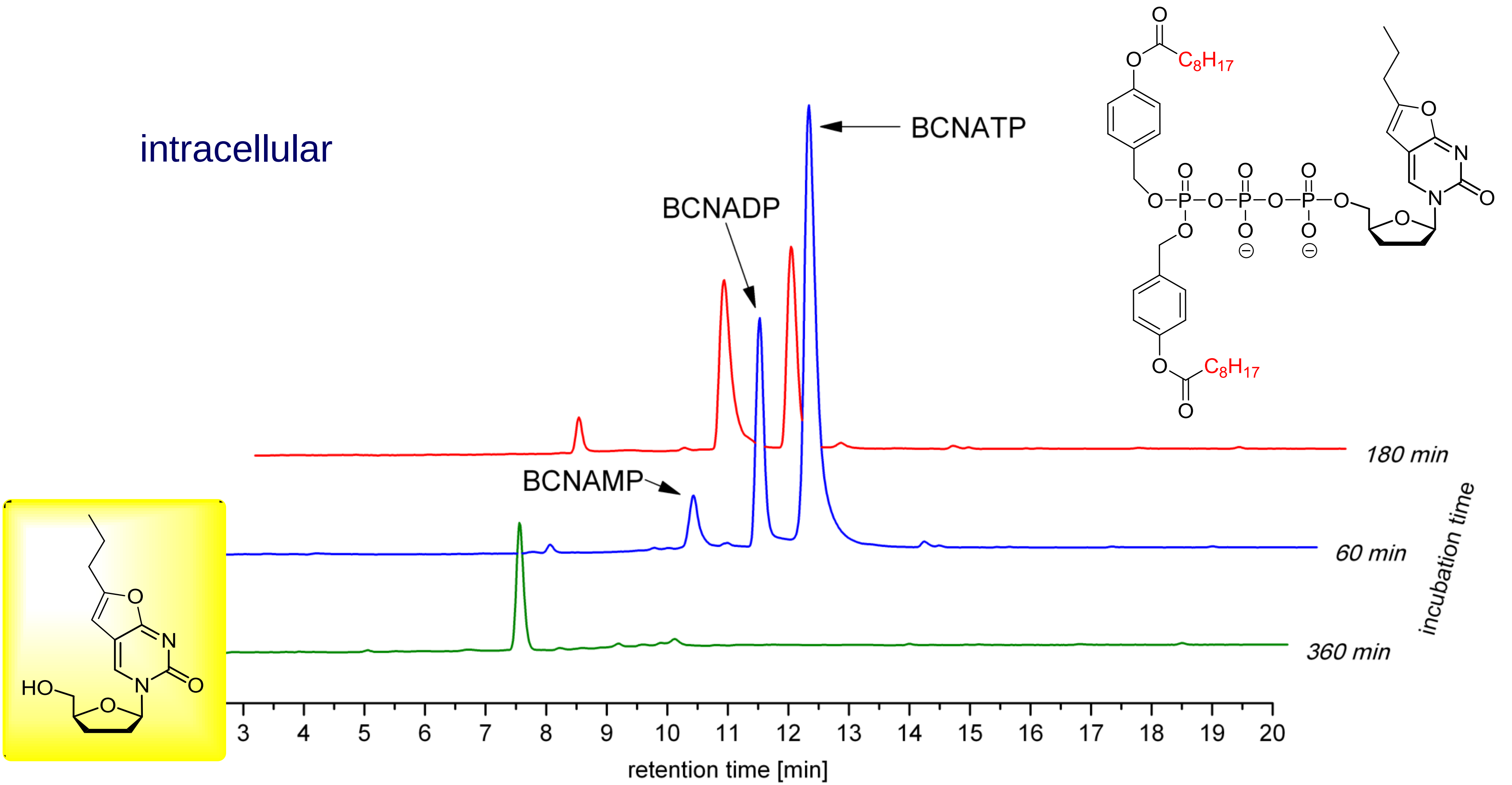
Lina Weinschenk



Non-symmetric vs. Symmetric Compounds



In-cell Delivery of a Triphosphate !



collaboration with I. Hauber, HPI, Hamburg

Summary

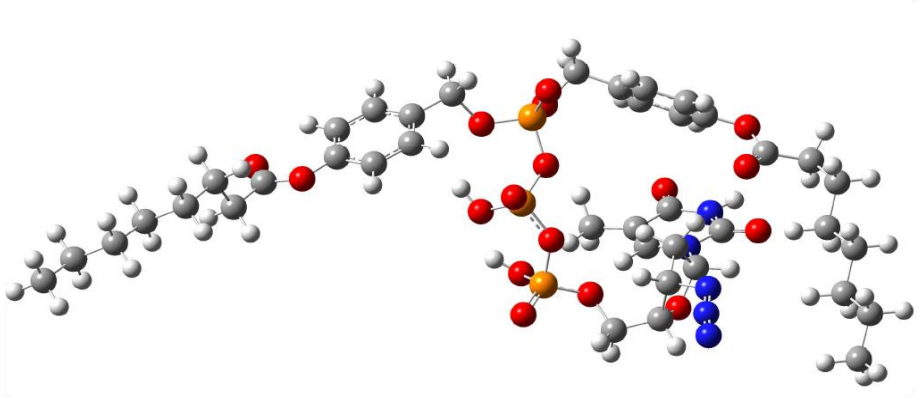
- Development of the first example of nucleoside triphosphate prodrugs

➡ **NO enzymatic phosphorylation of the nucleoside analogue is needed!**

- Chemical hydrolysis studies in PBS showed the predominant formation of NTP

- Enzymatic hydrolysis studies using PLE showed selective formation of NTP

- d4TDP- and d4TTP-prodrugs showed very good activity in thymidine kinases-deficient cells



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