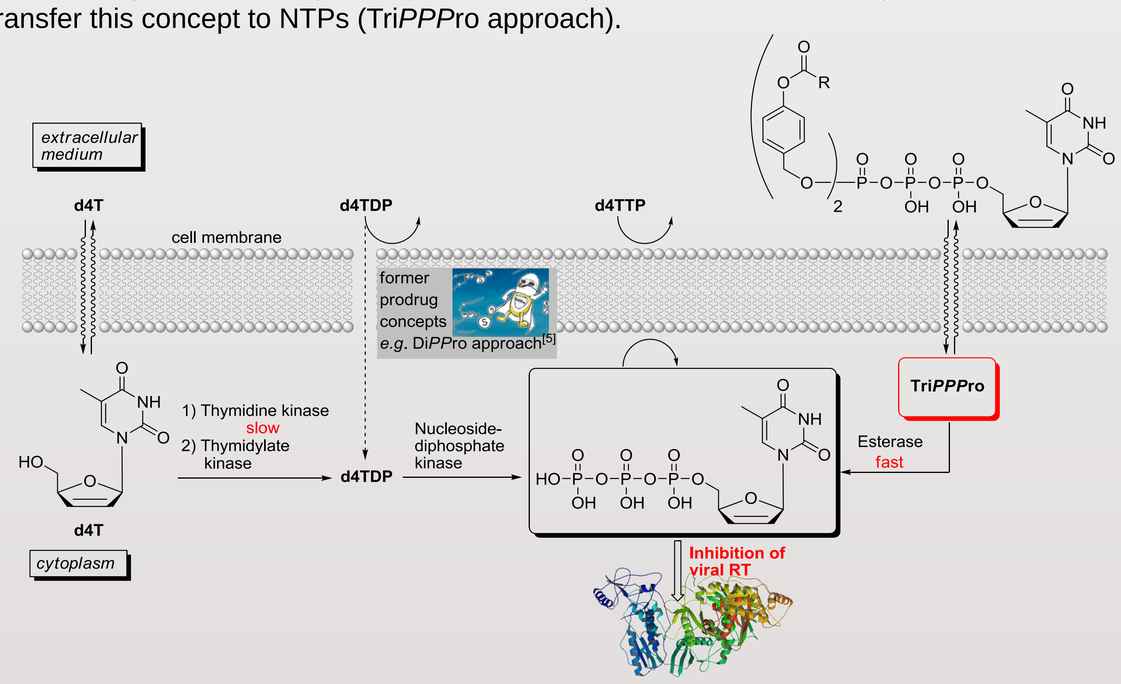
# Synthesis and Investigation of Potential Anti-HIV Active Nucleoside Triphosphate Prodrugs (Tri*PPP*ro-Compounds)


Tobias Nack[1], Dominique Schols[2], Jan Balzarini[2] and Chris Meier[1*]

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


## Introduction

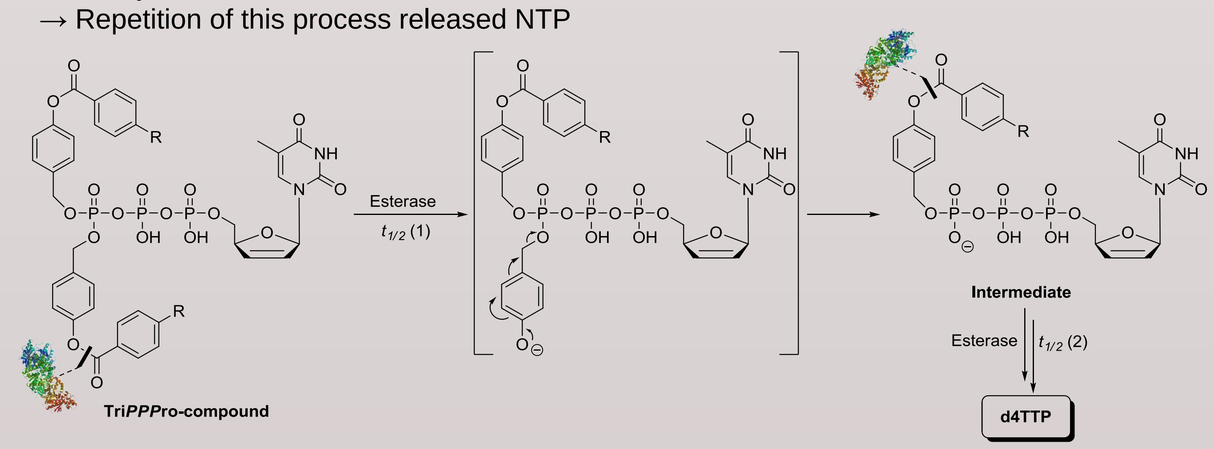
Nucleoside analogs are widely used for the treatment of antiviral infections and anticancer chemotherapy.[1] A limitation of these compounds is that they have to undergo biotransformation into the corresponding NTPs to act as inhibitors of the viral reverse transcriptase (RT).[2]
After cellular uptake of the nucleoside, this transformation is achieved *via* stepwise phosphorylation catalyzed by kinases (Scheme 1). Often, the first phosphorylation step is the bottleneck in the overall metabolism, *e.g.* for d4T. Nucleotide prodrugs represent a promising bypass to skip this processes. As a consequence of their lipophilic masking units they are able to penetrate through the cell membrane in contrast to the high negatively charged nucleotides.[3] Thus the biological activity of common nucleoside analogs has been improved and these prodrugs are valuable tools for studies regarding the nucleoside metabolism.[4]
Recently, we reported on the Di*PP*ro approach for the bioreversible protection of nucleoside diphosphates (NDP).[5] In contrast to the *cyclo*Sal approach[4], here the delivery mechanism relies on an enzymatically triggered process. Since a variety of nucleoside diphosphates with different aliphatic masking units have been synthesized and investigated, we were able to transfer this concept to NTPs (Tri*PPP*ro approach).




**Scheme 1:** Phosphorylation to the viral RT inhibitor d4TTP and the bypass by the Tri*PPP*ro approach.[6]

## The Tri*PPP*ro Hydrolysis Concept

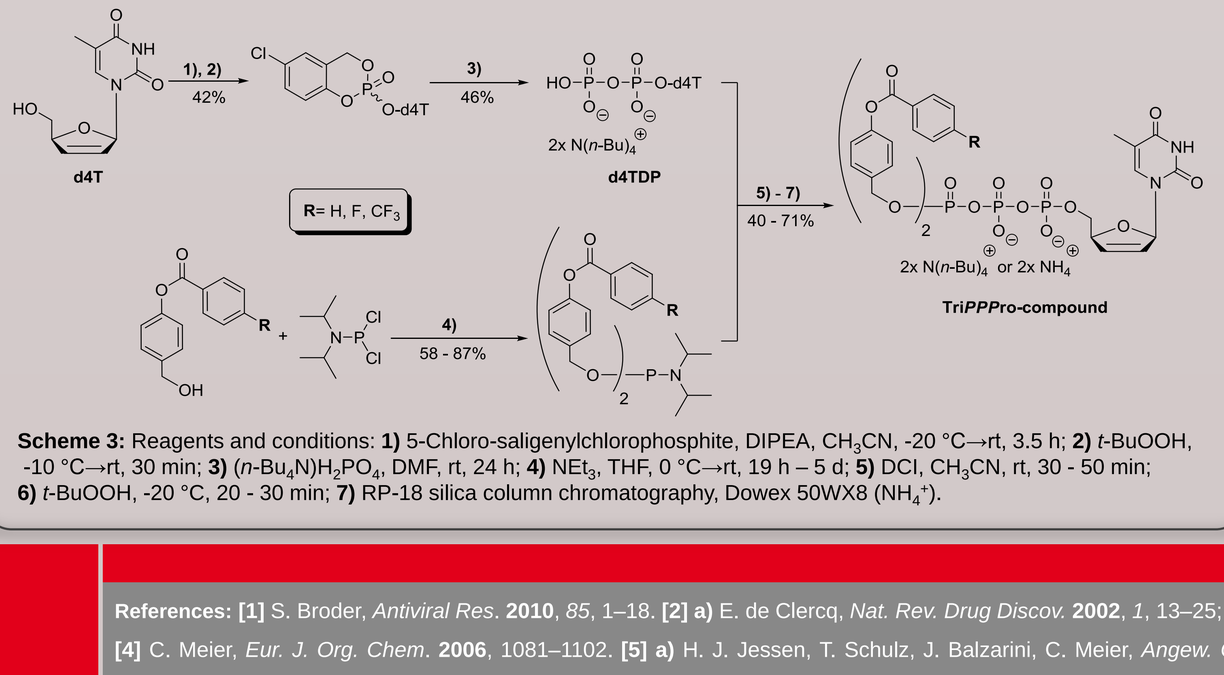
- Based on two acceptor substituted benzyl esters attached to the γ-phosphate
- Aroyl residues used as lipophilic masking units
- Upon enzymatic cleavage of the phenolic aroylester, a strong donor substituent was formed
  → benzyl bond is cleaved which led to an masked intermediate
  → Repetition of this process released NTP




**Scheme 2:** General structure and proposed hydrolysis pathway of aroyl-containing Tri*PPP*ro-compounds.[7]

## Synthesis

Starting with d4T, the nucleoside analog is stepwise phosphorylated to d4TDP using the *cyclo*Sal technology.[8] The corresponding Tri*PPP*ros were obtained by dicyanoimidazole (DCI) mediated coupling with bis(4-aroyloxybenzyl)phosphoramidites in yields up to 71%.




**Scheme 3:** Reagents and conditions: **1)** 5-Chloro-saligenylchlorophosphite, DIPEA, $CH_3CN$, -20 °C→rt, 3.5 h; **2)** *t*-BuOOH, -10 °C→rt, 30 min; **3)** $(n\text{-}Bu_4N)H_2PO_4$, DMF, rt, 24 h; **4)** $NEt_3$, THF, 0 °C→rt, 19 h – 5 d; **5)** DCI, $CH_3CN$, rt, 30 - 50 min; **6)** *t*-BuOOH, -20 °C, 20 - 30 min; **7)** RP-18 silica column chromatography, Dowex 50WX8 ($NH_4^+$).

## Proof of the Concept: Hydrolysis Studies

**In phosphate buffer (PBS):**

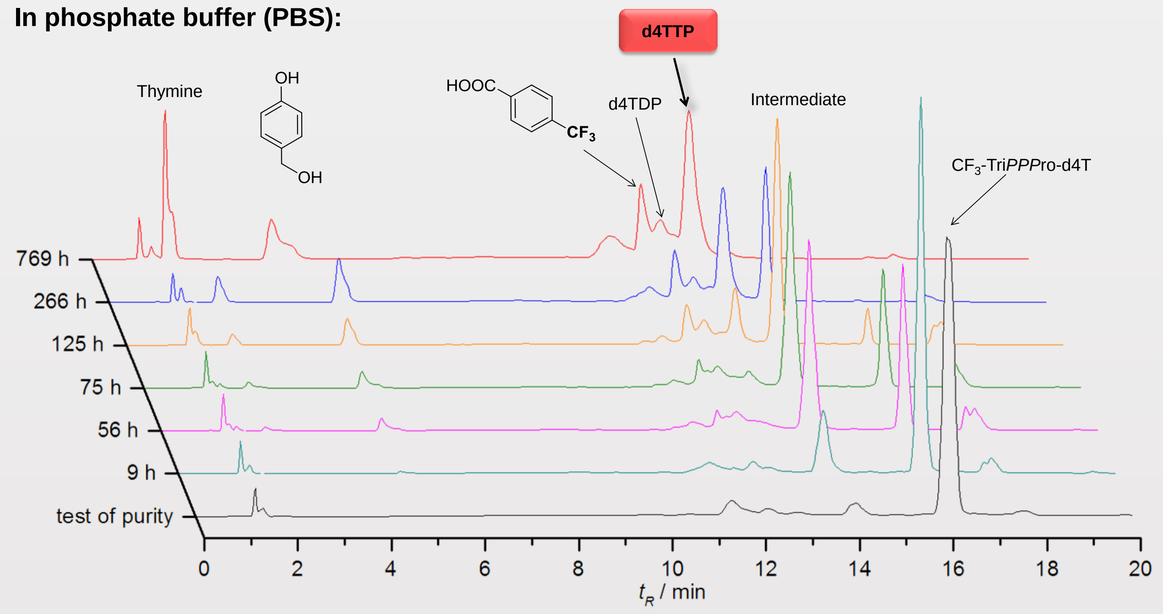



**Figure 1:** Ion-pair RP-HPLC profile of $CF_3$-Tri*PPP*ro-d4T after incubation in PBS, *pH*=7.3 (9-769 hours). Peaks were attributed by co-injection and/or $t_R$ of reference compounds.

**In PBS + porcine liver esterase (PLE):**

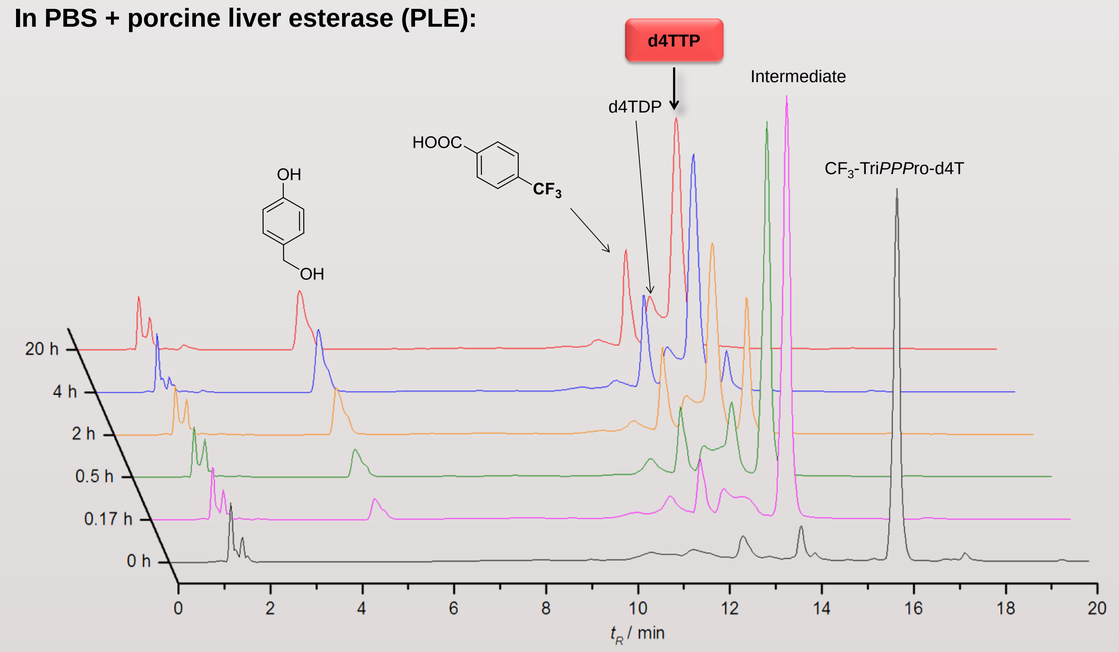



**Figure 2:** Ion-pair RP-HPLC profile of $CF_3$-Tri*PPP*ro-d4T after incubation in PBS with porcine liver esterase (PLE), 0.5 mg/mL, *pH*=7.3 (0–20 hours). Peaks were attributed by co-injection and/or $t_R$ of reference compounds.

**→ Highly selective formation of d4TTP in PBS and with PLE**

**→ Enzymatically triggered cleavage process accelerates hydrolysis**

## Hydrolysis Half-Lives and Antiviral Data

| | $t_{1/2}$ (Tri*PPP*ro-compounds) / h | | | $t_{1/2}$ (Intermediate) / h | |
|---|---|---|---|---|---|
| **R** | PBS [a] | CEM/0 [b] | PLE [c] | PBS [a] | PLE [c] |
| $CF_3$ | 18 ± 5 | 5.0 ± 0.7 | <10 min | 79 ± 10 | 1.1 ± 0.1 |
| F | 19 ± 4 | 7.9 ± 0.8 | <10 min | 320 ± 27 | 16.1 ± 1.7 |
| H | 23 ± 13 | 0.8 ± 0.4 | <15 min | 831 ± 59 | 22.0 ± 1.9 |

**Table 1:** First order hydrolysis half-lives of Tri*PPP*ro-compounds and intermediates in different media. a) phosphate buffer, *pH*=7.3, 50 mM; b) Human T-lymphocyte cell extract, *pH*=6.9; c) in PBS with porcine liver esterase, 0.5 mg/mL.

| | $EC_{50}$ / µM [a] MT-4 | | $CC_{50}$ / µM [b] MT-4 |
|---|---|---|---|
| **R** | HIV-1 | HIV-2 | |
| $CF_3$ | 0.22 | 0.56 | 100 |
| F | 0.38 | 0.81 | 60 |
| H | 0.46 | 0.59 | 85 |
| **d4T** | 0.50 | 0.83 | 176 |

**Table 2:** Antiviral data of Tri*PPP*ro-compounds and the parent nucleoside d4T. a) 50% Effective concentration; b) 50% Cytotoxic concentration.

## Conclusion

- Successful synthesis of different Tri*PPP*ro-d4T compounds in good yields
- Efficient release of d4TTP by cleavage of the bioreversible masking units
  → chemically stable, enzymatically labile
- easy tunable stability by changing the substitution pattern of the aroyl unit


**References:** **[1]** S. Broder, *Antiviral Res*. **2010**, *85*, 1–18. **[2] a)** E. de Clercq, *Nat. Rev. Drug Discov.* **2002**, *1*, 13–25; **b)** T. Cihlar, A.S. Ray, *Antiviral Res*. **2010**, *85*, 39–58. **[3]** C.R. Wagner, V.V. Iyer, E. J. McIntee, *Med. Res. Rev*. **2000**, *20*, 417–51. **[4]** C. Meier, *Eur. J. Org. Chem*. **2006**, 1081–1102. **[5] a)** H. J. Jessen, T. Schulz, J. Balzarini, C. Meier, *Angew. Chem. Int. Ed.* **2008**, *47*, 8719–8722; **b)** T. Schulz, J. Balzarini, C. Meier, *ChemMedChem* **2014**, *9*, 762–775. (For the „Di*PP*ro man" illustration see back cover of aforenamed *ChemMedChem* issue) **[6] a)** for the illustration of reverse transcriptase see www.rcsb.org/pdb, PDB ID: 1hmv **b)** D. W. Rodgers *et al*., *Proc. Natl. Acad. Sci.* USA **1995,** *92*, 1222–1226. **[7]** a) for the illustration of hCE1 see www.rcsb.org/pdb, PDB ID: 1yah **b)** C. D. Fleming, *et al., J. Mol. Biol.* **2005**, *352***,** 165–177. **[8]** S. Warnecke, C. Meier, *J. Org. Chem.* **2009**, *74*, 3024–3030.