Mono-, Di- and Triphosphate Prodrugs of the T-1105 Ribonucleotides

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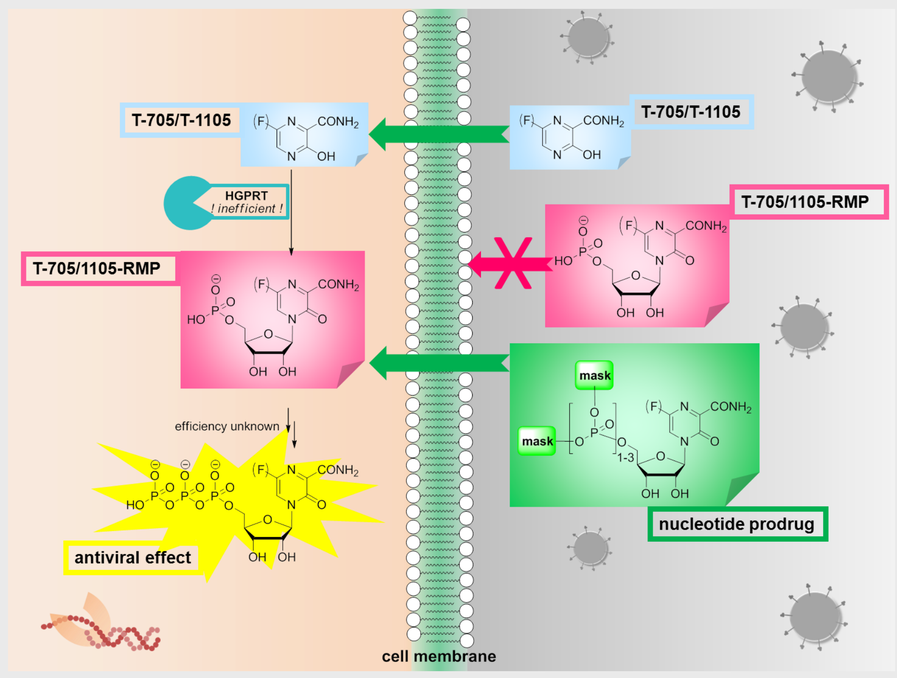
Introduction

- Nucleoside analogues (NAs) can interfere with RNA/ DNA replication → anticancer & antiviral agents.
- First: intracellular conversion to the nucleoside triphosphate.
- Often NAs are poor substrates for the phosphorylating enzymes → decreased therapeutic efficiency.

- T-705: heterocyclic carboxamide; developed to treat Influenza Virus infections; exhibits activity against a broad range of RNA viruses as does the defluoro analogue T-1105.
- It acts as a nucleobase analogue and is converted to the active ribosyl-triphosphate (RTP) inside cells:
  - First step: conversion of the heterocycle to the ribosyl-monophosphate (T-705-RMP), inefficiently catalyzed by the Hypoxanthine-Guanine-Phosphoribosyltransferase (HGPRT).[1]
  - Subsequent phosphorylation to the triphosphate most likely also catalyzed by host cell enzymes.

➡ Direct application of the nucleotides seems desirable to improve the antiviral activity.

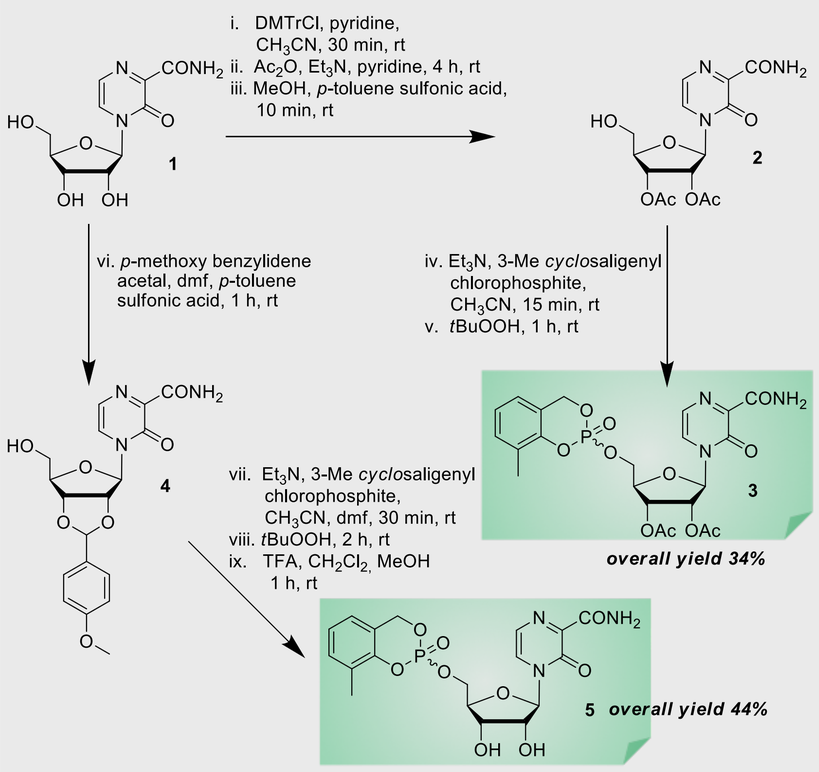


Objective

Intracellular delivery of the ribonucleoside mono-, di- and -triphosphate of T-1105 to improve the antiviral activities.

- **Synthesis of different Prodrugs**: mask the charges, increase the lipophilicity and release the nucleotide inside the cell.
  - Nucleoside Monophosphate: ***cyclo*Sal-concept**[2]
  - Nucleoside Diphosphate: **Di*PP*ro-concept**[3]
  - Nucleoside Triphosphate: **Tri*PPP*ro-concept**[4]

Synthesis and antiviral Evaluation of the *cyclo*Sal-Pronucleotides



- Successful synthesis of the 2',3'-di-*O*-acetyl-protected and the 2',3'-unprotected 3-Me-*cyclo*Sal-T-1105-RMP-pronucleotides - first ever reported T-1105-RMP-pronucleotides.

- New and highly efficient synthesis of *ribo*-*cyclo*Sal-pronucleotides employing the *para*-methoxy benzylidene protecting group.

  - For unknown reasons, both pronucleotides **3** and **5** didn't show significant antiviral activity when studied against EBOV, LASV and CCHFV in Vero cells while the parent compound proved to potently inhibit virus replication.

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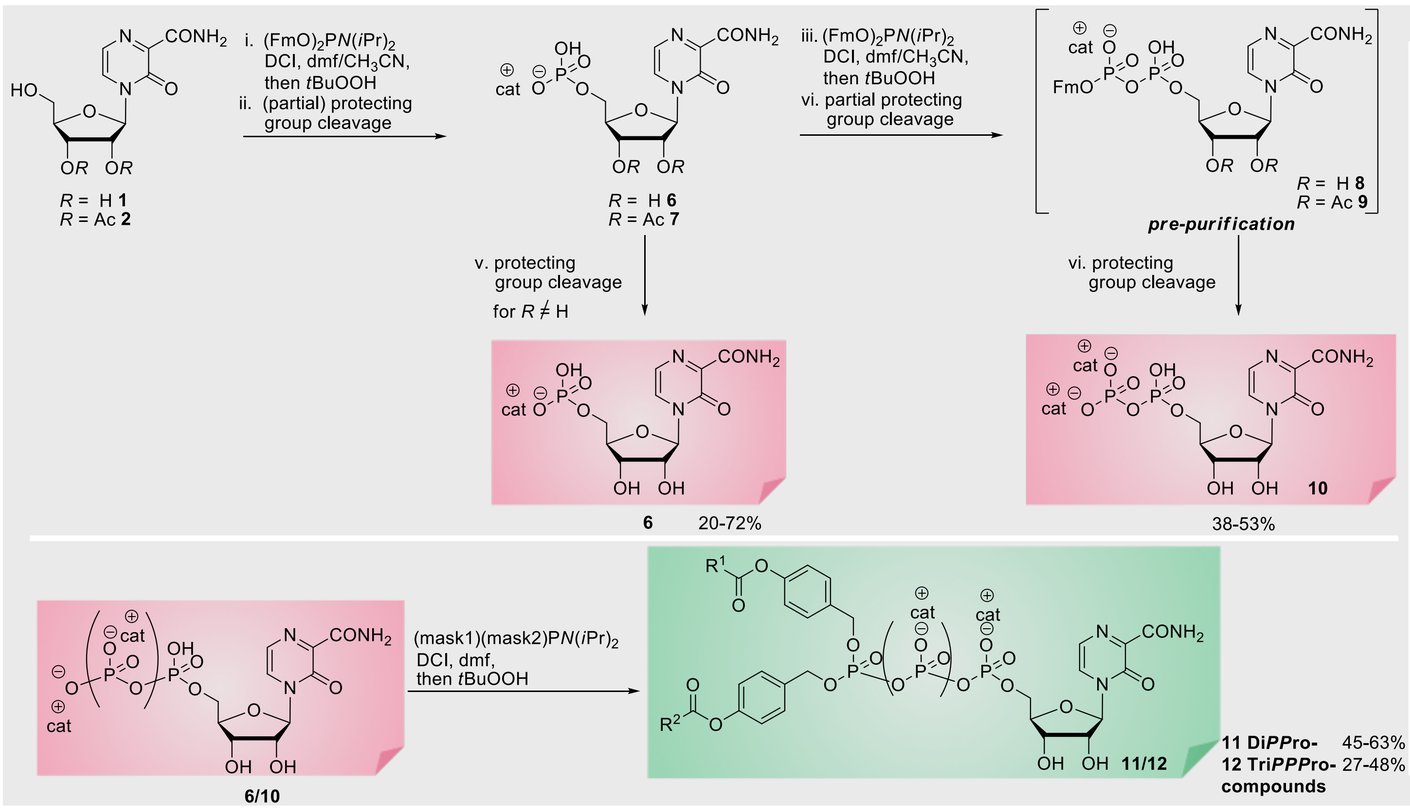
Intracellular release of T-1105-RMP to be studied.

Synthesis and antiviral Evaluation of the Di*PP*ro- and Tri*PPP*ro-Compounds



- First report on the synthesis of T-1105-RMP **6** and -RDP **10**. Modification of the bis(fluorenylmethyl) phosphoramidite protocol for the DP synthesis[5] led to DP in excellent purity.

- Successful synthesis of Di*PP*ro-T-1105-RDPs and Tri*PPP*ro-T-1105-RTPs in high purities - first ever reported di- and triphosphate prodrugs of the T-1105 riboside.

  - The Di*PP*ro- and Tri*PPP*ro-compounds **11**/**12** showed moderate *in vitro* activities against CCHFV.

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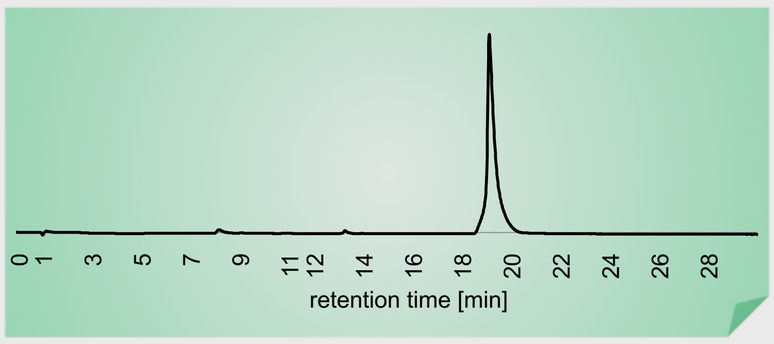
Next: 1) Studies on the stability of the compounds;
2) Studies in different cell lines and mouse model.



RP-HPLC-chromatogram (shown for R^1=R^2=C_9H_{19}-Di*PP*ro **11**; absorption at 350 nm) confirms 98% purity after four freeze-thaw-cycles in dmso.

Conclusion and Outlook

➡ Successful synthesis of mono-, di- and triphosphate prodrugs of T-1105 ribonucleotides.

➡ Antiviral evaluation against three different hemorrhagic fever viruses shows no activity for the MP prodrugs but moderate activity for the Di*PP*ro- and Tri*PPP*ro-compounds.

➡ Do the prodrugs penetrate the cell membrane and release the respective nucleotide?

References

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