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Lability of the Favipiravir Ribonucleoside and First Mechanistic Details

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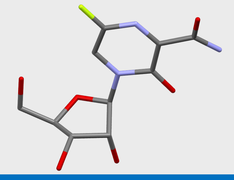


✔ Reliable chemical synthesis

→ Instable towards nucleophiles

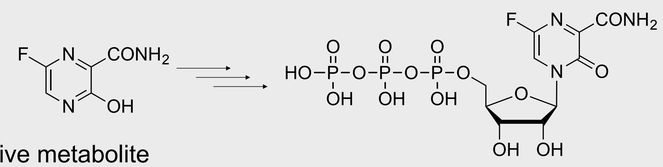
→ Instable in aqueous media

→ Nucleophilic substitution of Fluorine, followed by a decomposition of the pyrazine

✚ Biological implications?

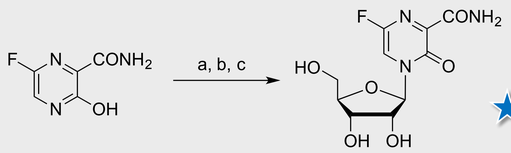
T-705 (6-fluoro-3-hydroxy-2-pyrazinecarboxamide; favipiravir)

- Fluorinated *N*-aromatic
- Activities against numerous RNA viruses
- T-705-ribonucleoside 5'-triphosphate has been described to be the active metabolite



Chemical Synthesis of T-705-Ribonucleoside

- Vorbrueggen nucleoside synthesis followed by standard deacetylation protocols resulted in decomposition.
- Optimization of the glycosylation and deacetylation provided a **reliable protocol for this synthesis:**

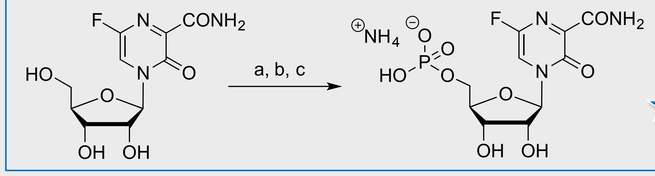


a. Hexamethyldisiloxane, $(NH_4)_2SO_4$, 60 min, 140 °C
b. Tetra-*O*-acetyl-β-D-ribofuranose, $SnCl_4$, acetonitrile, 7 h, rt
c. Bu_2SnO, methanol, 24 h, 80 °C
overall yield: 40%.

★ Focus on the deacetylation:
→ Avoid strong nucleophiles and bases

Chemical Synthesis of T-705-Ribonucleoside 5'-Monophosphate

- The Sowa and Ouchi protocol was adapted to the specific demands of the T-705-ribonucleoside:
  - Short reaction and hydrolysis time
  - Careful neutralization to pH 7 with an aqueous ammonium bicarbonate solution

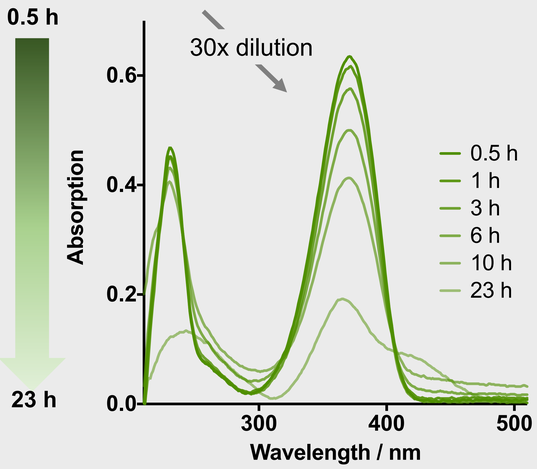


a. phosphorus oxychloride, pyridine, water, acetonitrile, 3 min, 0 °C
b. addition of T-705-ribonucleoside, 20 min, 0 °C
c. NH_4HCO_3 solution to pH 7.0
overall yield: 50%.

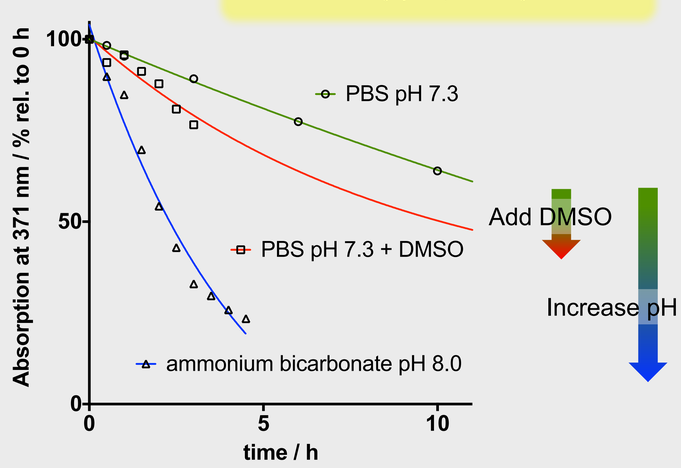
★ Focus on the neutralization:
→ Avoid (local) pH > 7

UV/Vis Spectroscopy

- T-705-ribonucleoside (3 mM) in phosphate buffer (pH 7.3, 50 mM, +4% CH_3CN) at 37 °C.

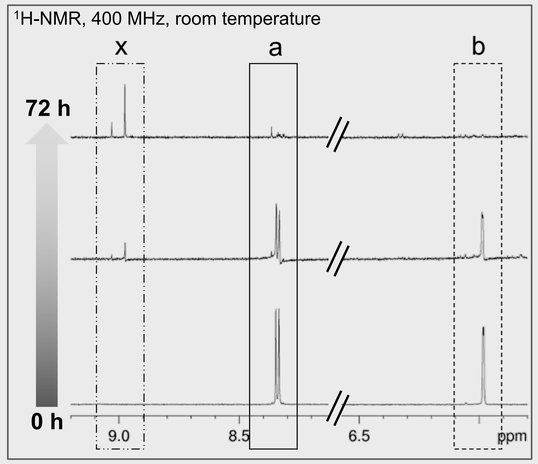


→ The chromophore decomposes, this is accelerated by pH ↑ and by + DMSO



NMR Spectroscopy

- T-705-ribonucleoside in *deuterated* phosphate buffer (pH 7.8, 200 mM, room temperature).



→ β-glycosidic proton resonance (b) disappears

→ aromatic proton resonance (a) disappears

→ singlet (x) at approx. 9 ppm emerges

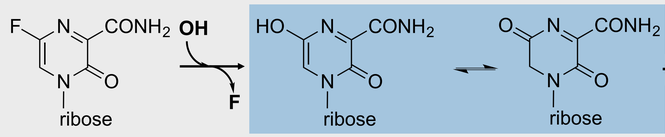
simultaneous decomposition of the aromatic pyrazine derivative and the glycosidic bond

Mass Spectrometry

- T-705-ribonucleoside in **a)** H_2O *or* **b)** $H_2{}^{18}O$ (3.5 mM), 37 °C.

➤ HR-ESI-MS

a) *m/z* 288.0826 - $[(C_{10}H_{13}N_3O_7)+H]^+$	**a)** *m/z* 328.0754 - $[(C_{10}H_{15}N_3O_8)+Na]^+$
b) *m/z* 290.0871 - $[(C_{10}H_{13}N_3O_6{}^{18}O)+H]^+$	**b)** *m/z* 332.0838 - $[(C_{10}H_{15}N_3O_6{}^{18}O_2)+Na]^+$
➡ Fluorine is displaced by a hydroxyl group	➡ then, water is added



decomposition of the aromatic system

Conclusion

✓ T-705 – while *N*-glycosidically bound to ribose – proved highly labile towards nucleophilic displacement of the Fluorine substituent.

✓ This leads to a fast decomposition of the nucleobase analogue in various aqueous media that are indispensable in biochemical assays.

→ Further studies on stabilized ribonucleoside analogues & on the biological implications!

References This study was published in: J. Huchting, M. Winkler, H. Nasser, C. Meier *ChemMedChem* **2017**, *12*, 652–659.

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