Inhibition de 2'*O*- et *N*7-méthyltransférases virales par des nucléosides et oligonucléotides bi-substrats

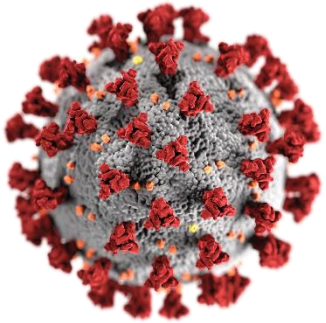
Rostom AHMED-BELKACEM, PhD

Séance – Académie Nationale de Pharmacie
8 février 2023

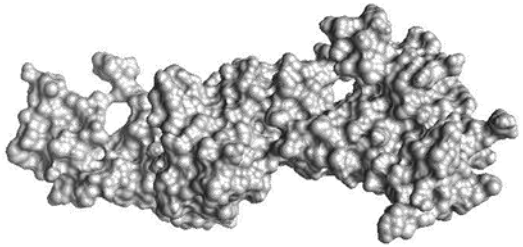
Institution de rattachement :
Institut des Biomolécules Max Mousseron, Université de Montpellier

Emerging viruses → RNA viruses

- **SARS-CoV-2** ⟶ **Covid-19**



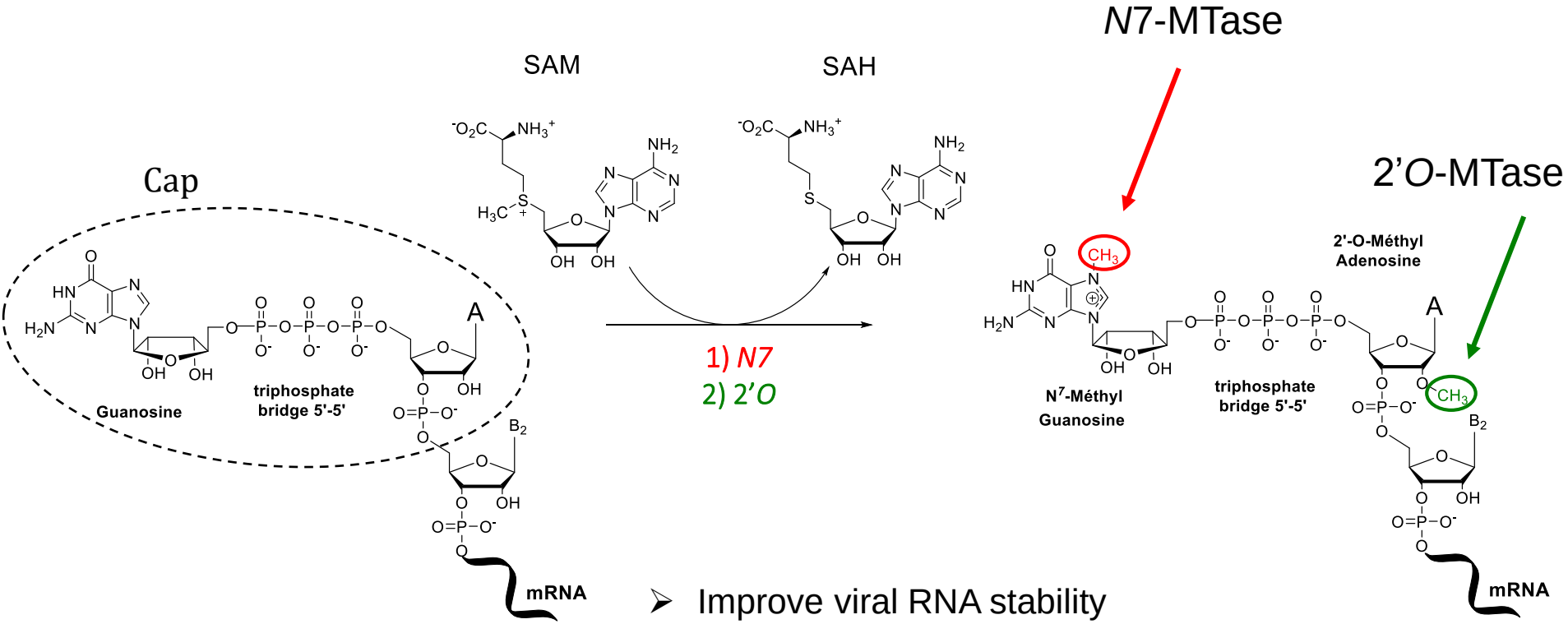
January 2019 : **Inhibition of a viral protein of SARS-CoV (2002 – 2003) !**



But we must not forget…

- ❑ **Ebola**
- ❑ **Dengue**
- ❑ **Zika**
- ❑ **Chikungunya**

mRNA cap methylation after infection

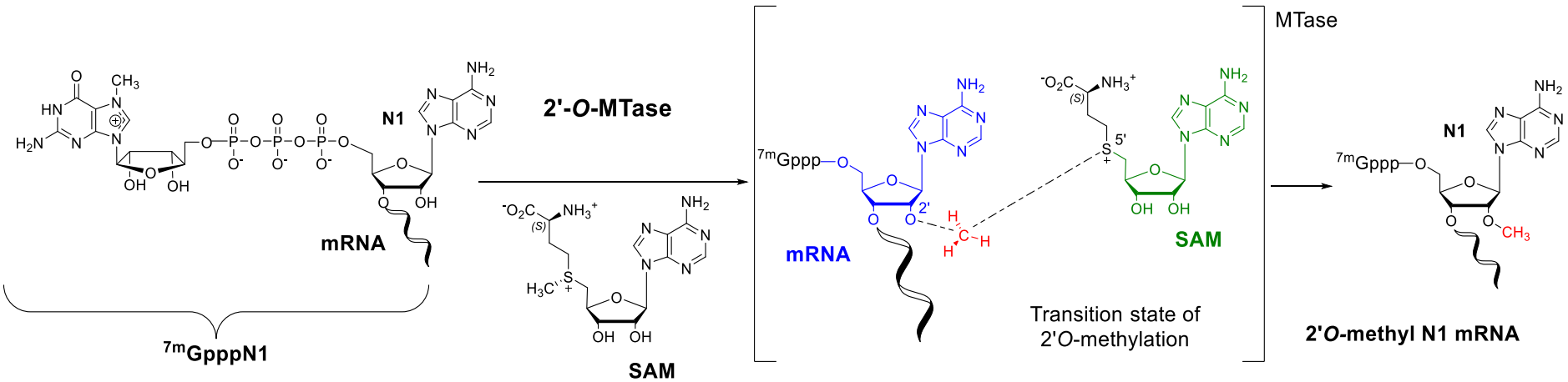


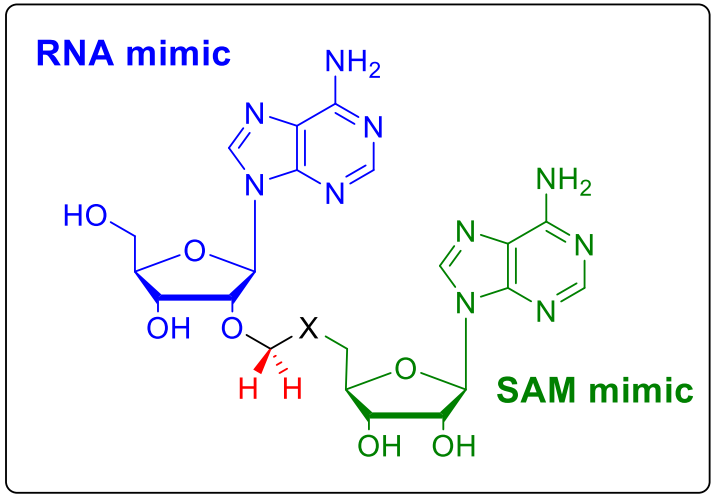
- Improve viral RNA stability
- Improve translation of mRNA by ribosomes
- Hide and protect mRNA against immune system

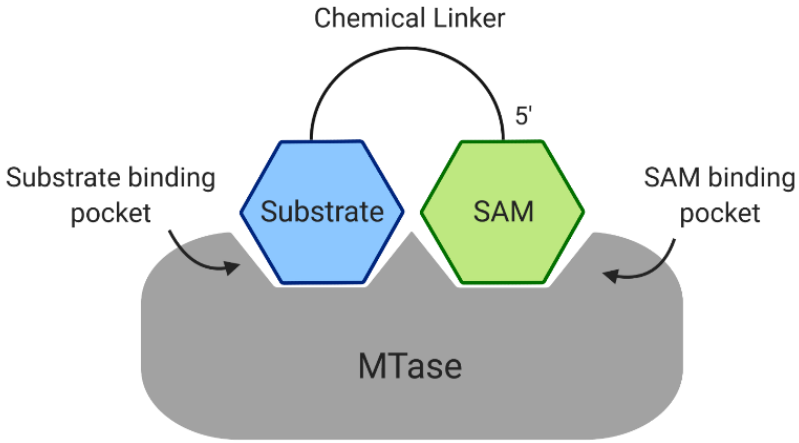
Blocking methylations : Strategy to inhibit viral infection

Design of SAM/mRNA bisubstrate inhibitors

2'*O*- transition state mimics

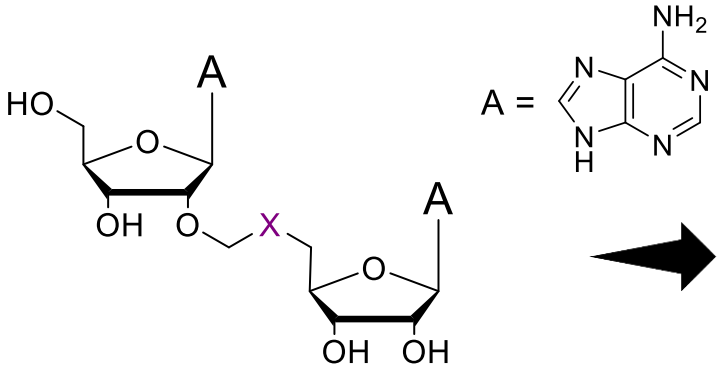






Bisubstrate Strategies to Target Methyltransferases
Ahmed-Belkacem, R.; Vasseur, J. J.; Debart, F., *Eur. J. Org. Chem.* 2022

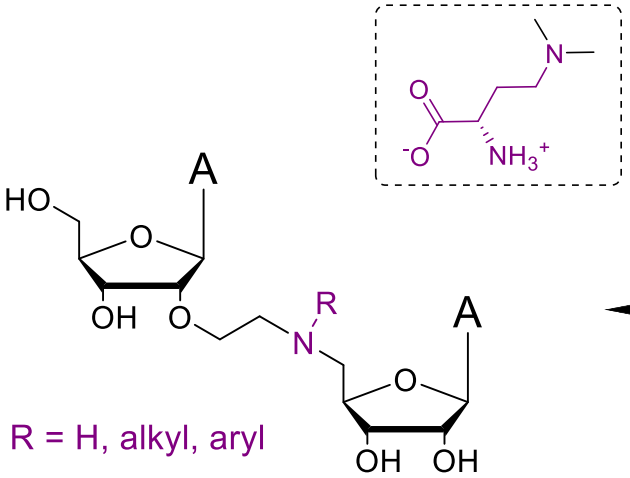
1st series : *S*-Dinucleosides mRNA/SAM analogs (10/2017 – 11/2018)



X = S, SO, SO_2, CH_2-S, S-S

Ahmed-Belkacem, R.; Sutto-Ortiz, P.; Decroly, E.; Vasseur, J. J.; Debart, F., *Eur. J. Org. Chem.* **2019**, 6486-6495.

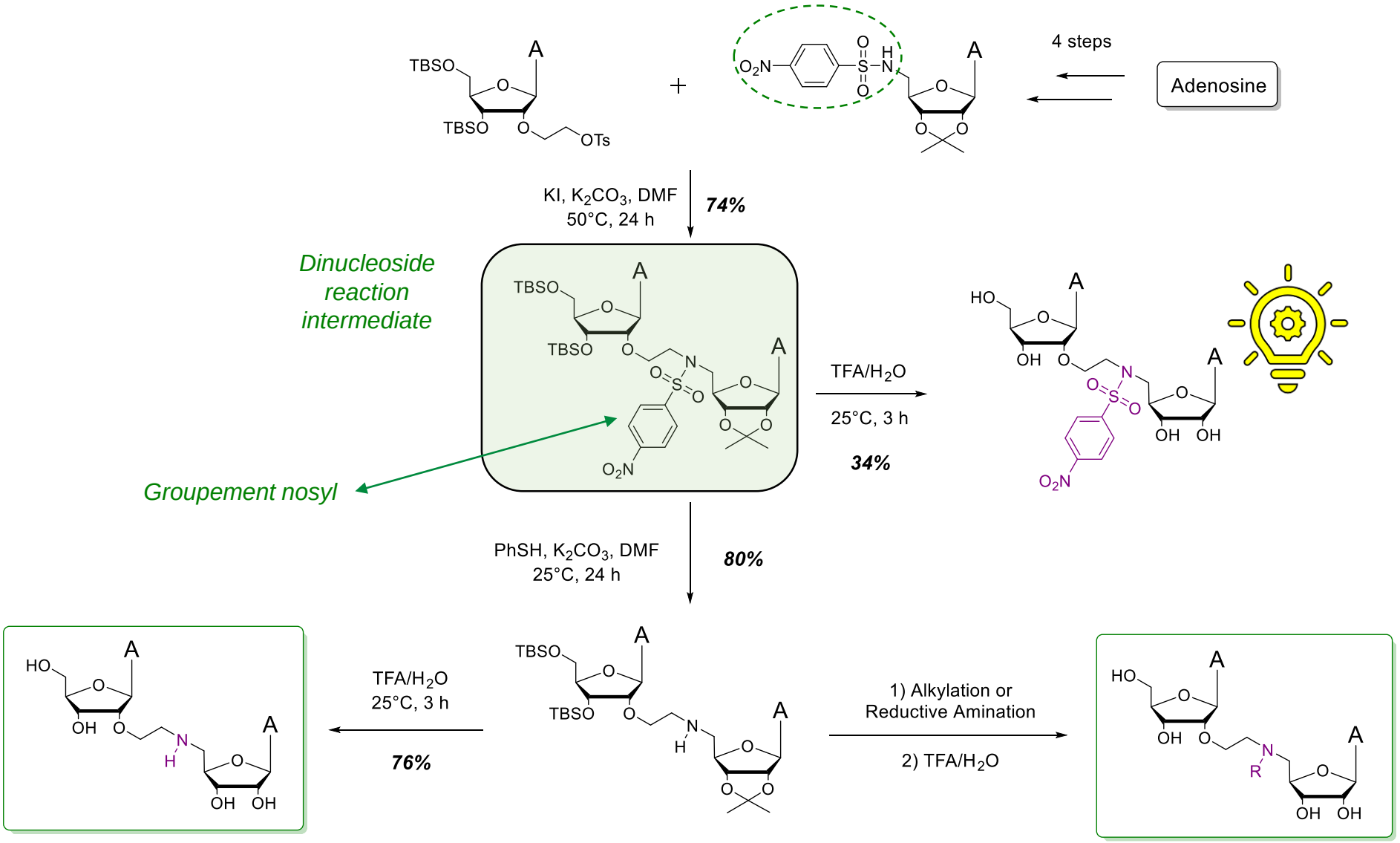
2nd series : *N*-Dinucleosides mRNA/SAM analogs (10/2018 – 12/2019)



R = H, alkyl, aryl

Ahmed-Belkacem, R.; Sutto-Ortiz, P.; Guiraud, M.; Canard, B.; Vasseur, J.-J.; Decroly, E.; Debart, F., *Eur. J. Med. Chem.* **2020,** *201*, 112557.

Synthesis Strategy



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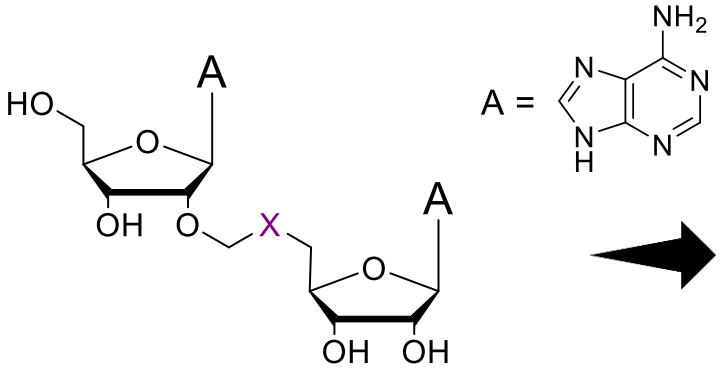

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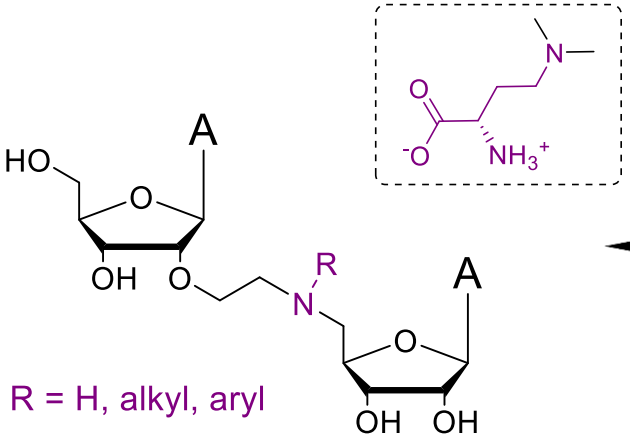
Ahmed-Belkacem, R.; Sutto-Ortiz, P.; Decroly, E.; Vasseur, J. J.; Debart, F., *Eur. J. Org. Chem.* **2019**, 6486-6495.

No inhibition of 2'*O*-MTases !

2nd series : *N*-Dinucleosides mRNA/SAM analogs (10/2018 – 12/2019)



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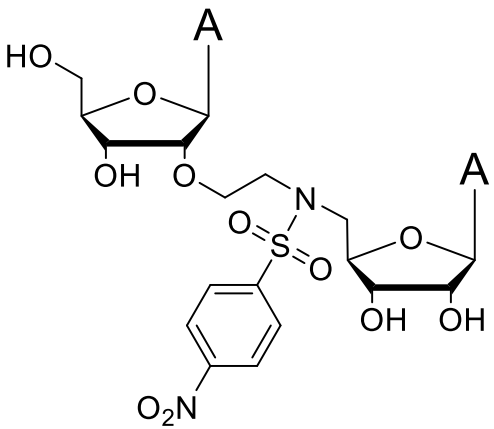
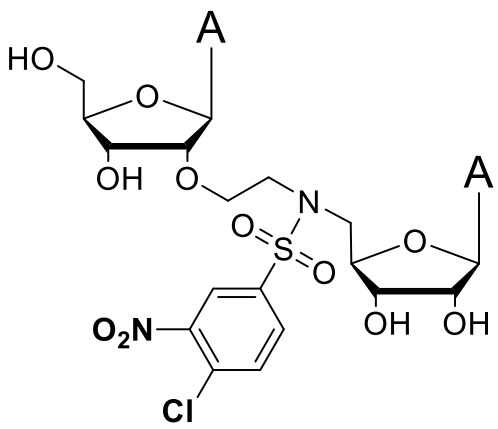
No inhibition of 2'*O*-MTases !

An unexpected result (SARS-CoV)

Inhibition of SARS-CoV nsp14 (*N*7-Mtase)



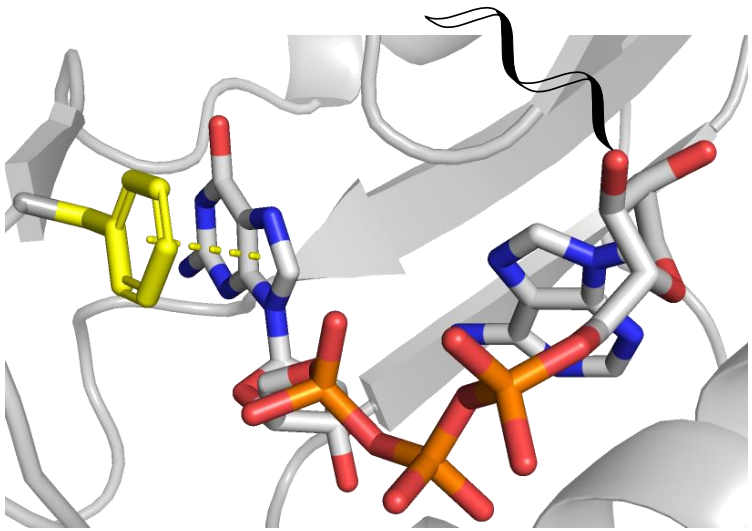


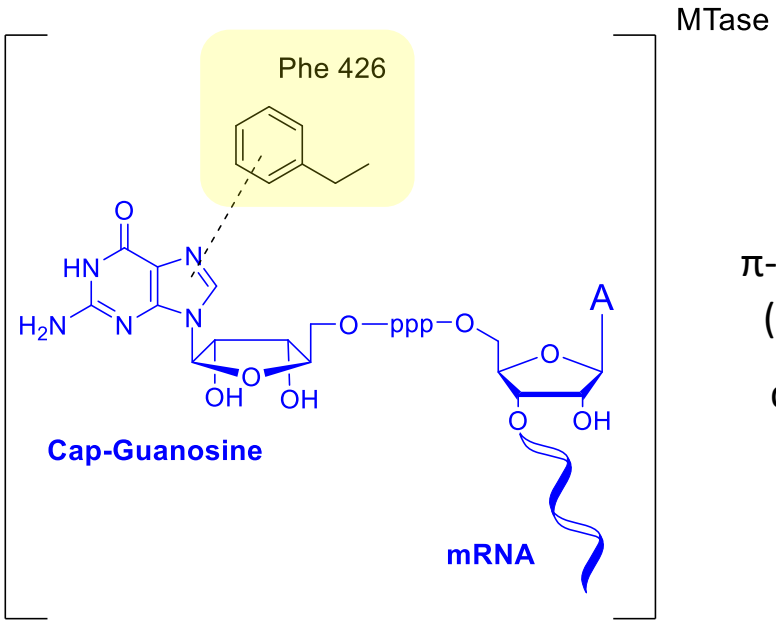
	IC$_{50}$ – First compound	
SARS-CoV	nsp14	2.6 µM
Human	hRNMT	> 500 µM
Vaccinia	D1/D12	> 500 µM

IC$_{50}$ – Optimized compound	
nsp14	0.6 µM
hRNMT	248 µM
D1/D12	> 500 µM

Molecular Docking

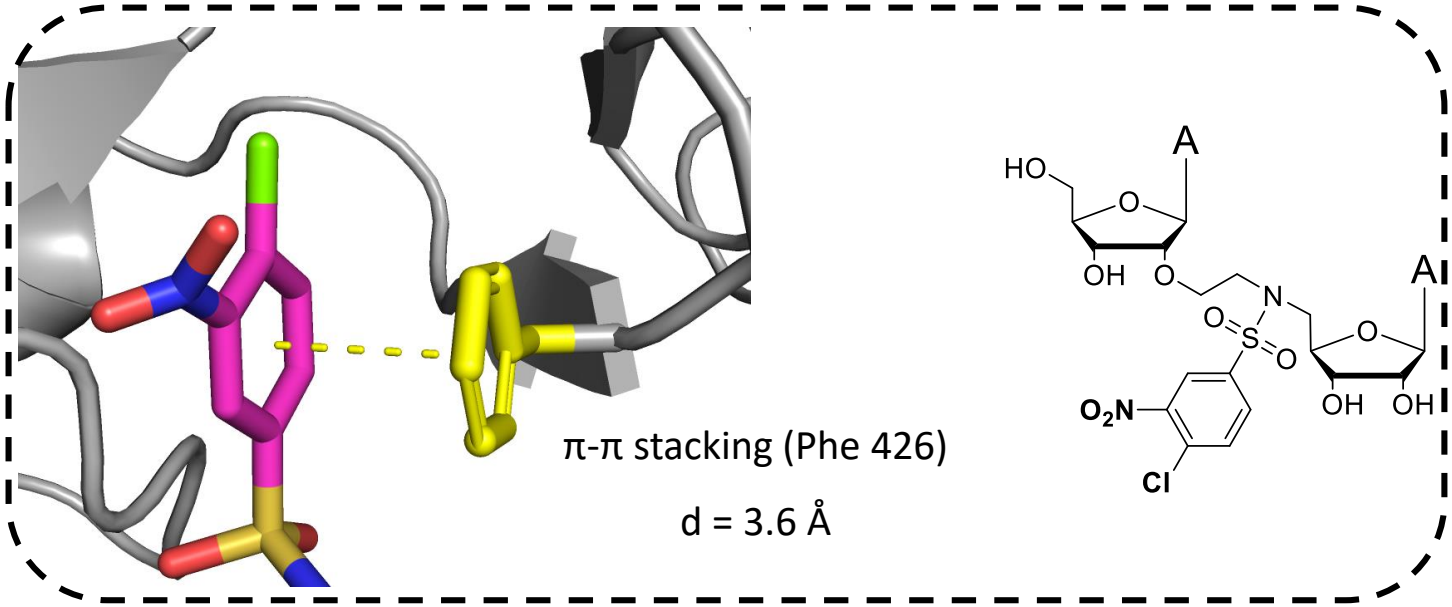
Naturally, PHE426 interacts with viral mRNA





π-π stacking
(Phe 426)

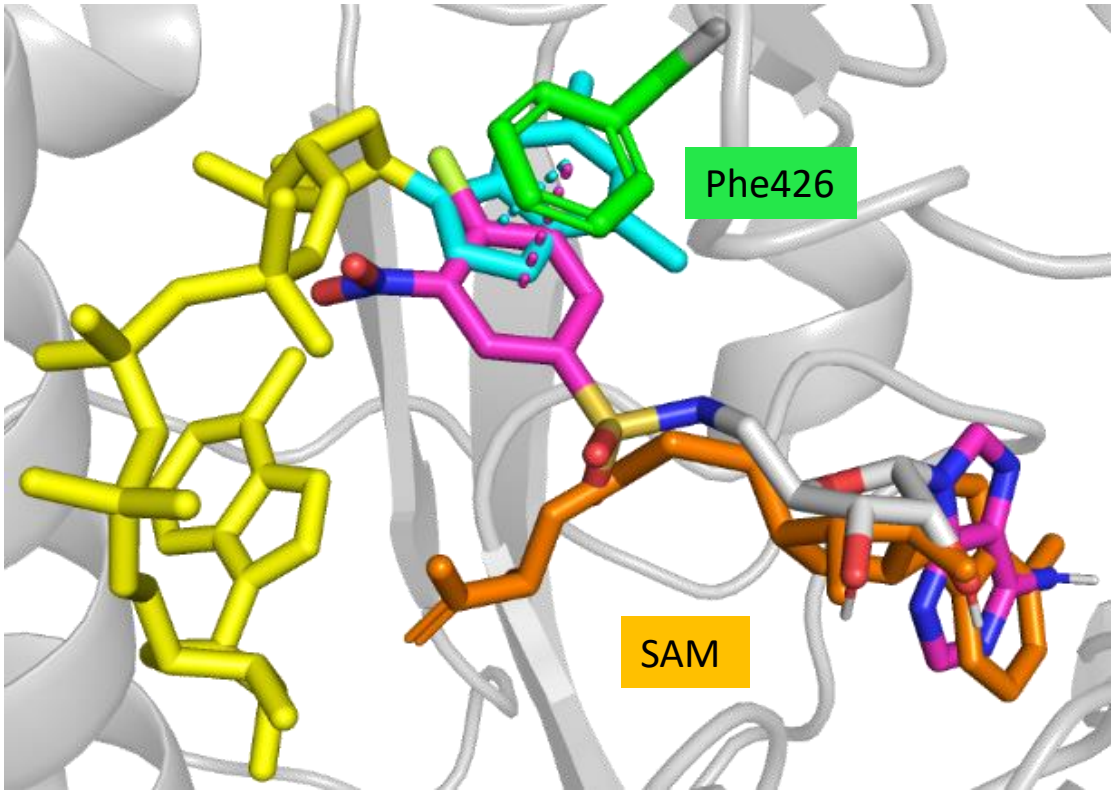
d = 3.7 Å



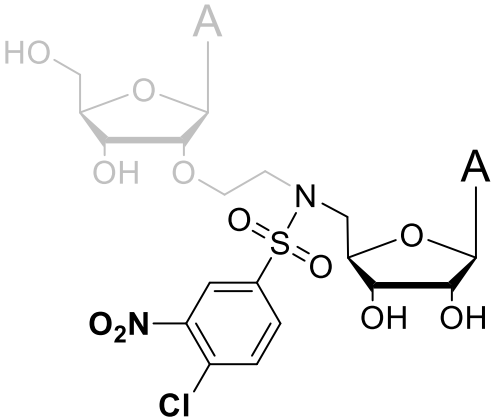
Molecular Docking

Guanosine (Coiffe)

ARNm

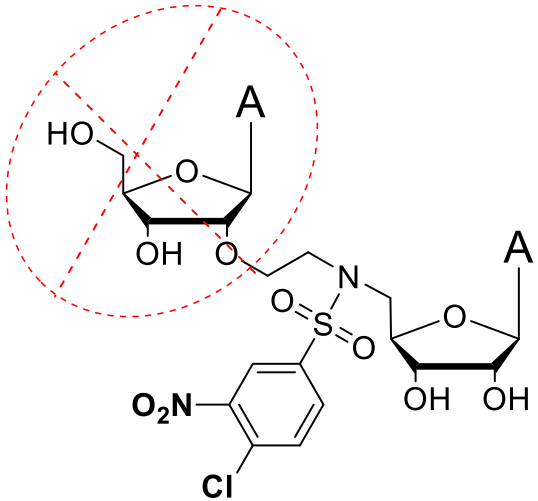


Not shown for quality purpose

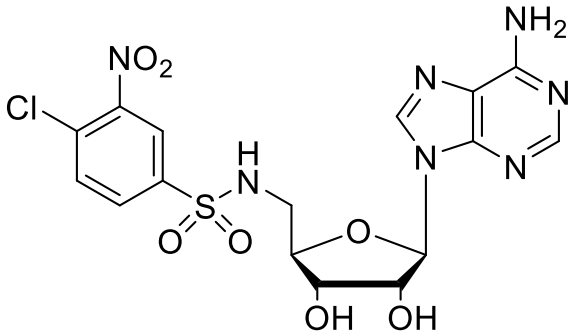


Bisubstrates Inhibitors **SAM**/**Cap-guanosine**

Inhibition of SARS-CoVs nsp14 (N7-Mtase)

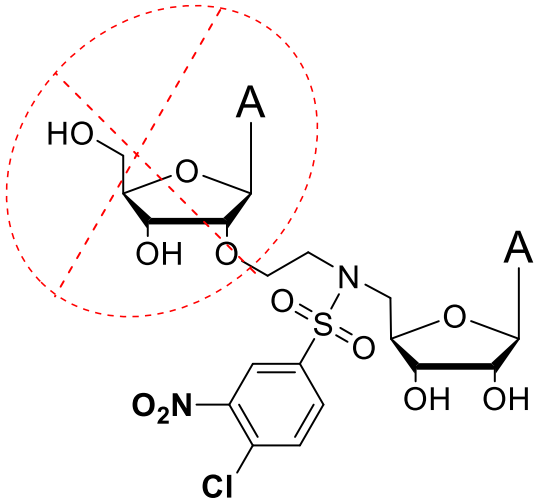


IC_{50} SARS-CoV nsp14 = 0.6 µM

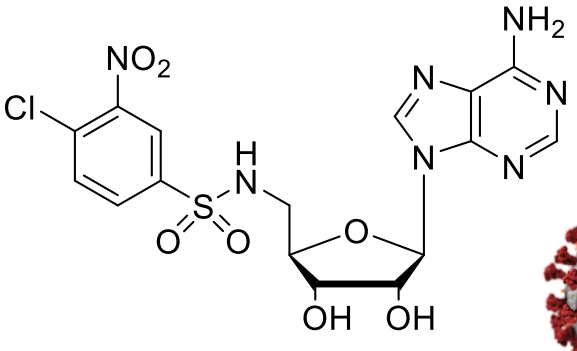


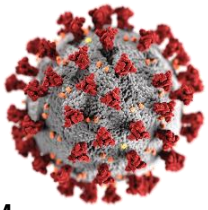
IC_{50} SARS-CoV nsp14 = 1.4 µM

Inhibition of SARS-CoVs nsp14 (N7-Mtase)



IC_{50} SARS-CoV nsp14 = 0.6 µM





IC_{50} SARS-CoV nsp14 = 1.4 µM

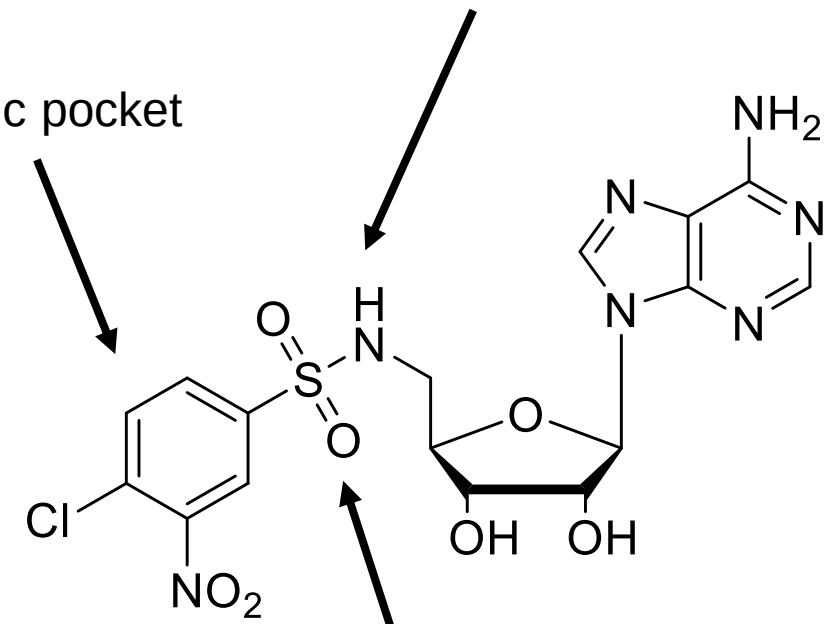
IC_{50} SARS-CoV-2 nsp14 = 2.1 µM

Optimize inhibition of SARS-CoV-2 nsp14 (*N*7-MTase)

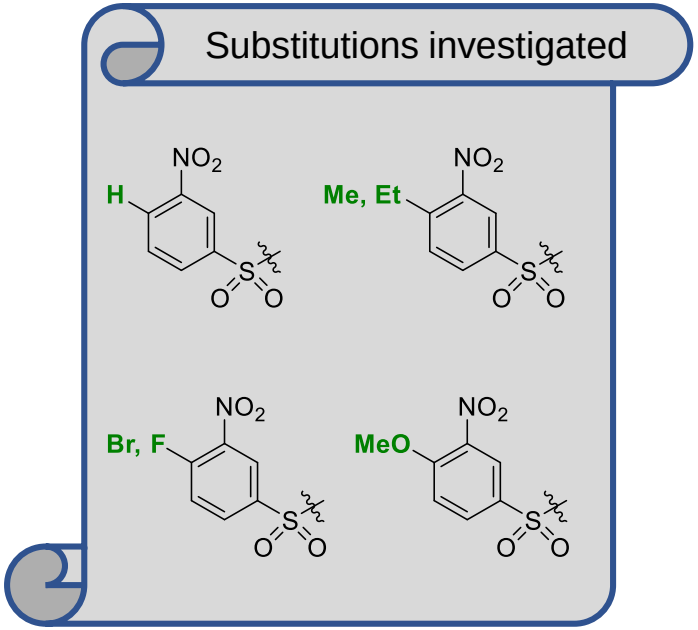
Several modification sites that have all been studied,

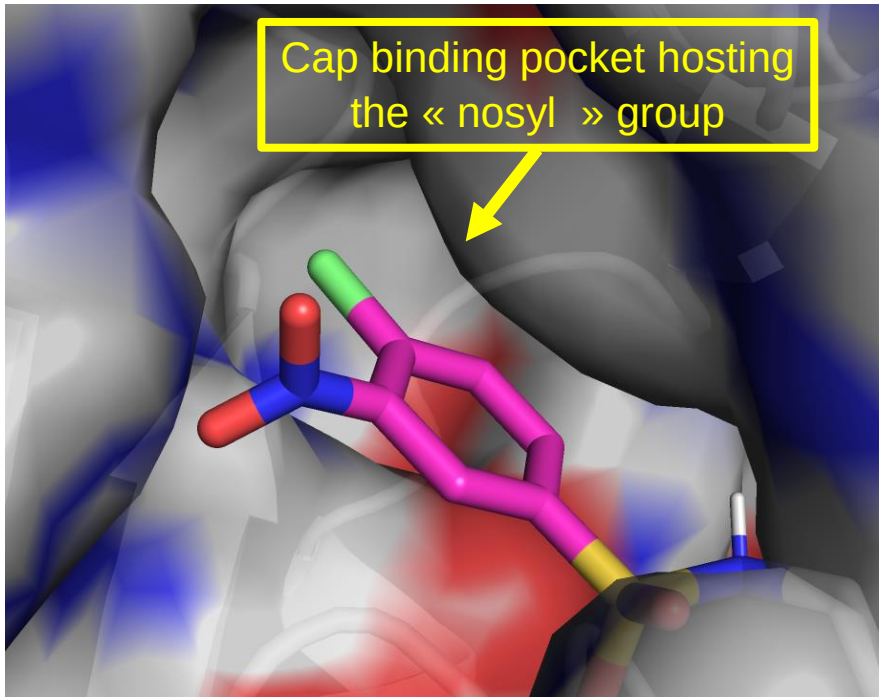
For this talk:

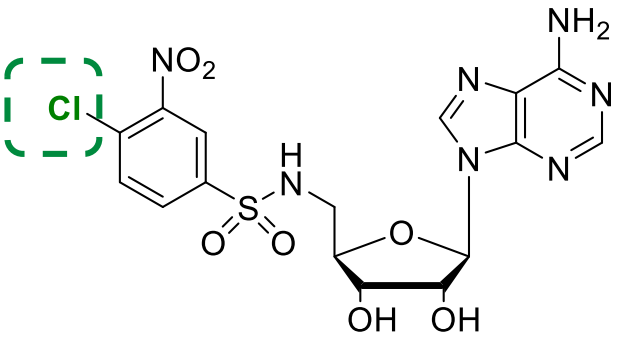
Potent Inhibition of SARS-CoV-2 nsp14 N7-Methyltransferase by Sulfonamide-Based Bisubstrate Analogues. J. Med. Chem. ***2022****.* https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c00120



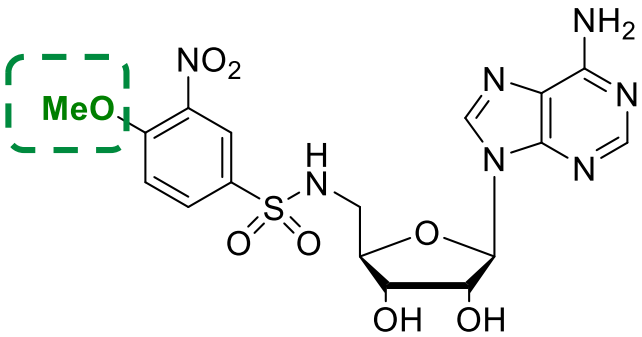
Replacing p-Cl by small groups (H, F, OMe…)





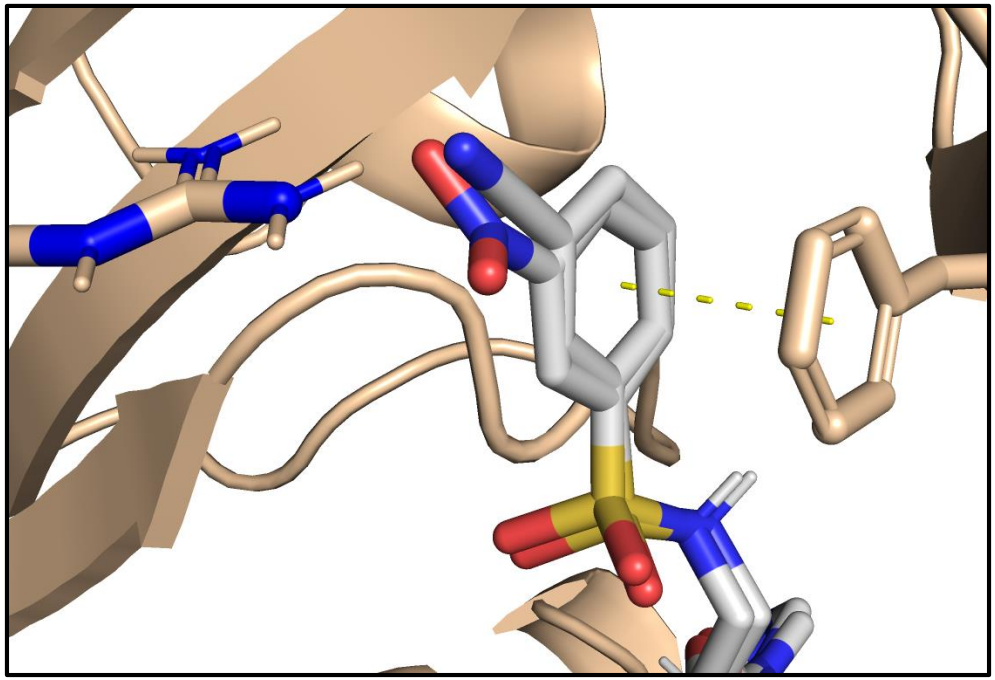


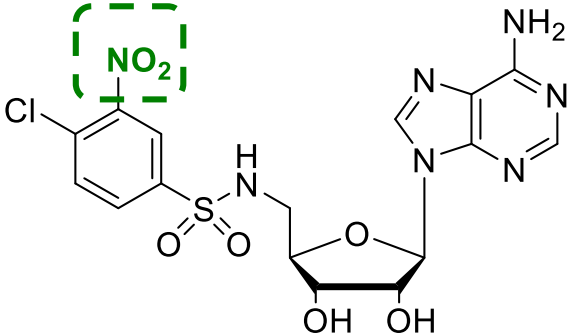
IC_{50} SARS-CoV-2 nsp14 = 2.6 µM

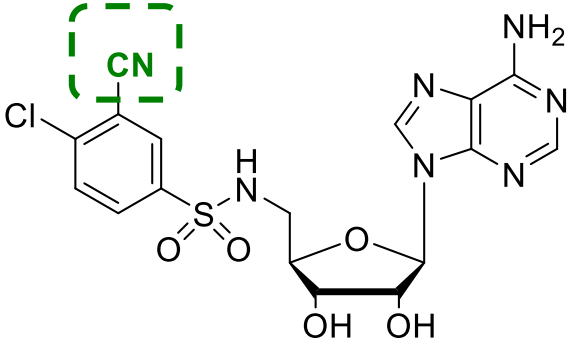


IC_{50} SARS-CoV-2 nsp14 = 0.146 µM

Replacing m-NO_2 by an other electro attracting group: m-CN





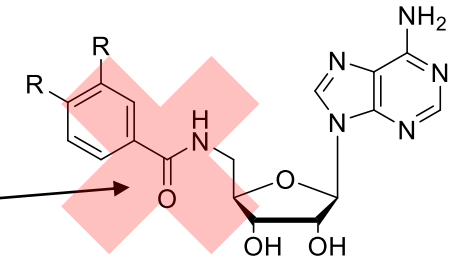


IC_{50} SARS-CoV-2 nsp14 = 2.6 µM

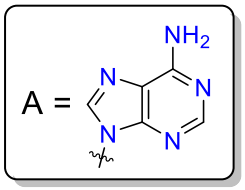
IC_{50} SARS-CoV-2 nsp14 = 1.0 µM

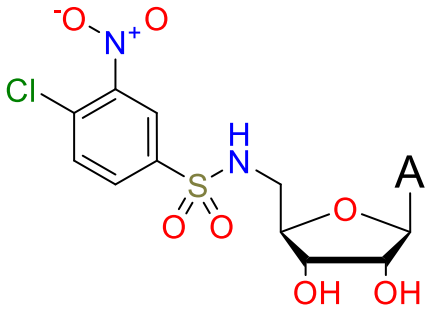
Novel Inhibitors of SARS-CoV-2 nsp14 - Summary

- ≈ 40 synthesized and tested compounds
  - ≈ 20 among them with IC_{50} < 1 µM
  - Linkage: Sulfonamide better than Amide (no inhibition)
  - Addition of ethyl group on N-atom improve inhibition



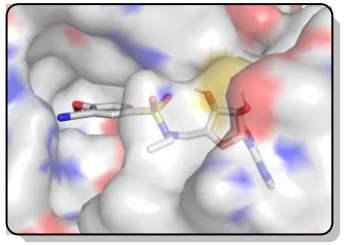
First nucleoside





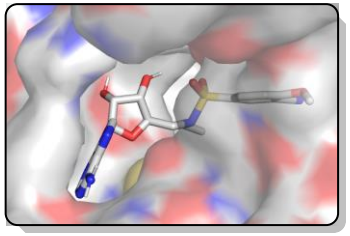
IC_{50} SARS-CoV nsp14 = 1.4 µM
IC_{50} SARS-CoV-2 nsp14 = 2.1 µM

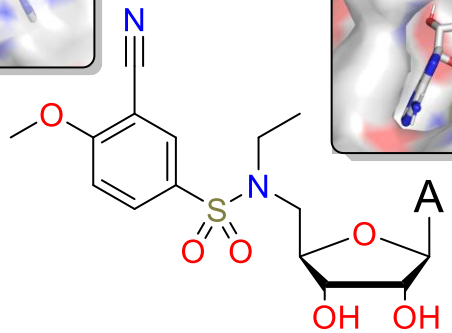
Cap Binding Site



Optimized nucleoside

SAM Binding Site





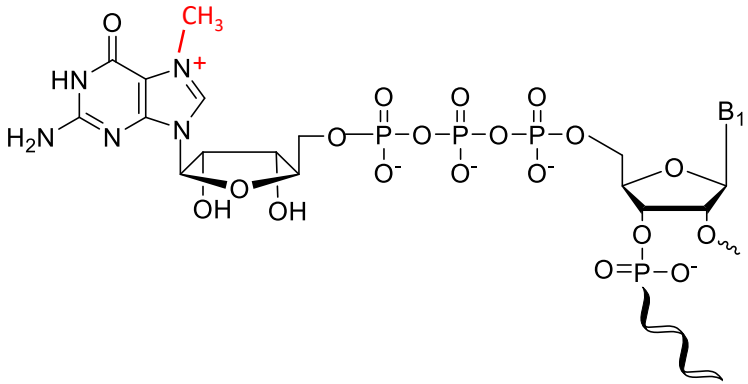
IC_{50} (SARS-CoV-**2** nsp14) = 0.019 µM
IC_{50} (hRNMT) = 53 µM ; SI > 2000

Inspired from graphical abstract in

Potent Inhibition of SARS-CoV-2 nsp14 N7-Methyltransferase by Sulfonamide-Based Bisubstrate Analogues. J. Med. Chem. ***2022****.*
https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c00120

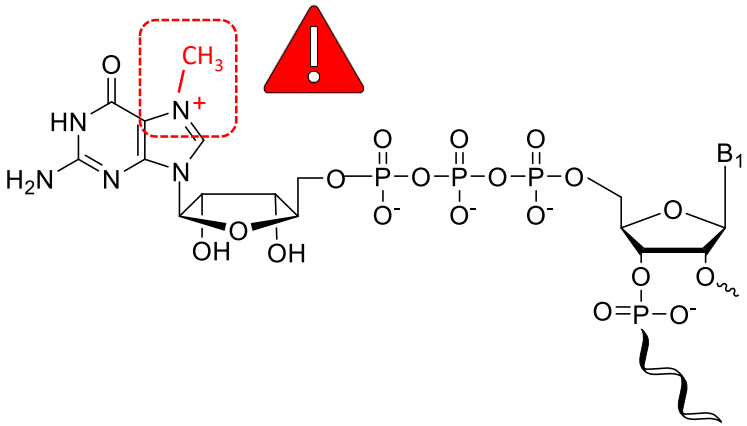
Absence of 2'*O*-MTase recognition ? Cap-dependant enzymes

NS5 ZIKV/DENV



J. Virol. **2017**, *91* (5)

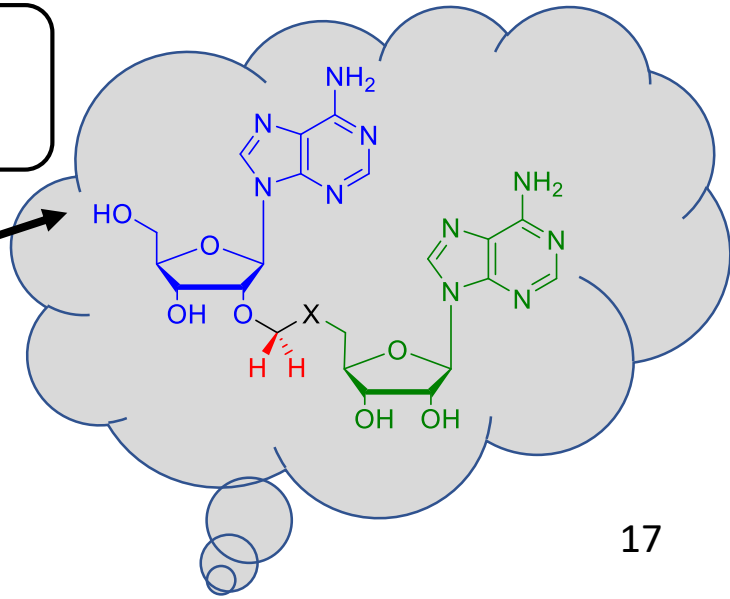
SARS-CoV & SARS-CoV-2 nsp 16



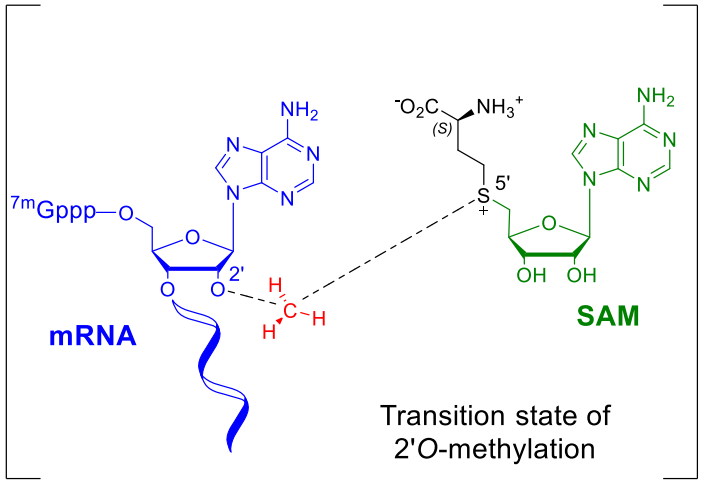
J. Virol. **2008**, *82* (16)

NS5 et nsp16 are **cap-dependant** 2'*O*-MTases

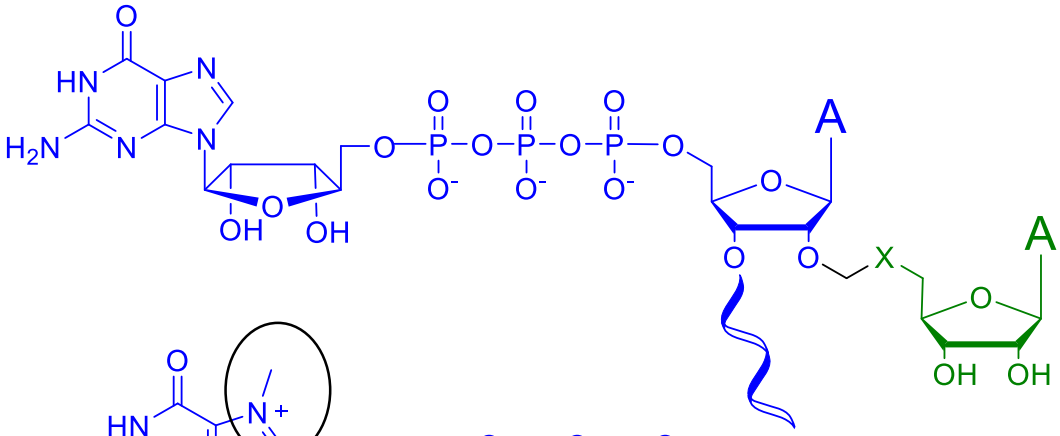
5' extremity : no cap-structure

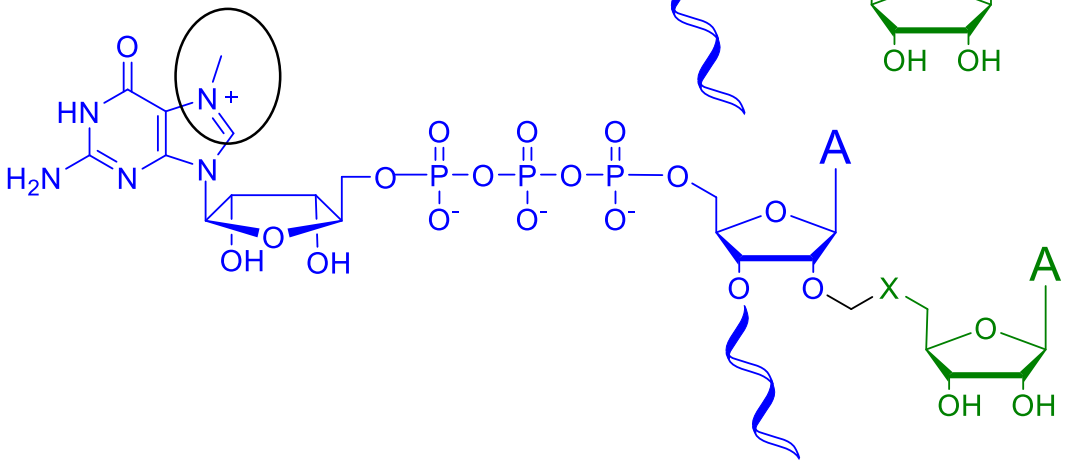


Construction of oligoribonucleotides (ORN) mRNA/SAM



Oligonucleotide synthesizer (µmol scale)

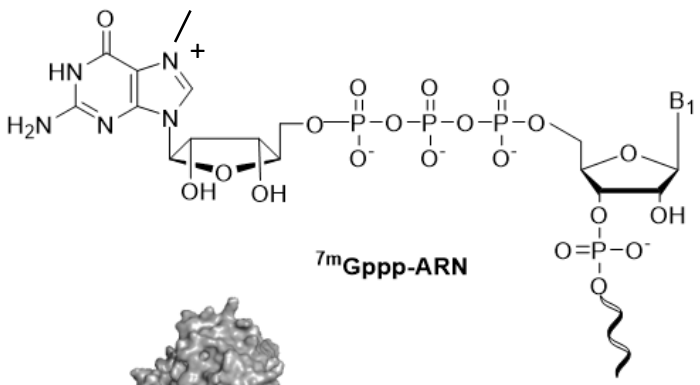


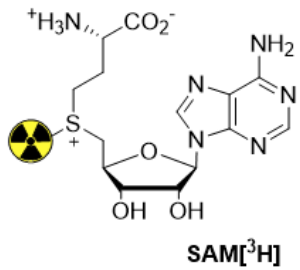


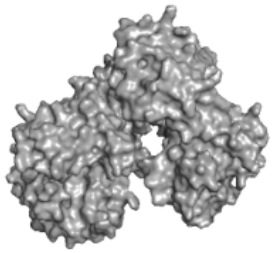
<u>Viral RNA sequence</u>

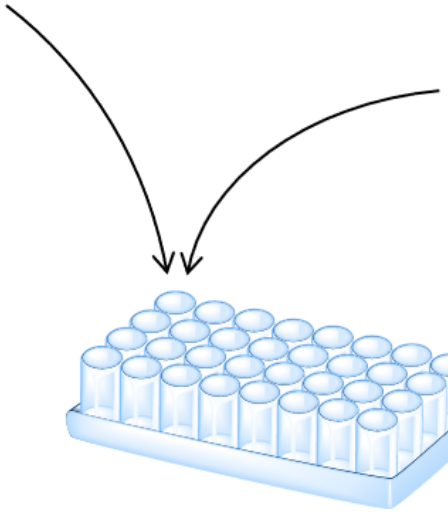
Dengue/Zika : 5'-**A**GUUGU-3'
SARS-CoV-2 : 5'-**A**UUAAA-3'

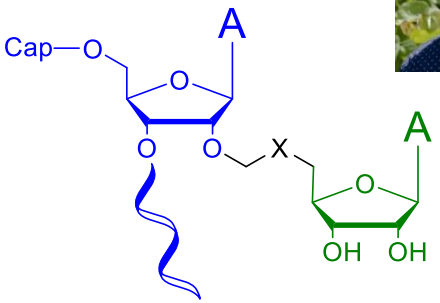
Competitive Inhibition Assay on 2'*O*-MTase











Inhibition of 2'*O*-MTases !

AFMB

Viral Replicases team :
Structure, function and drug-design

Dr. B. Canard

Dr. E. Decroly

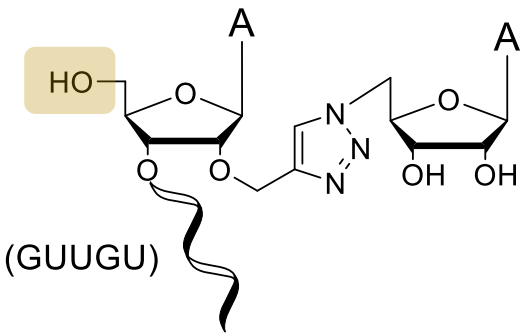
Dr. P. Sutto-Ortiz

A. Delpal

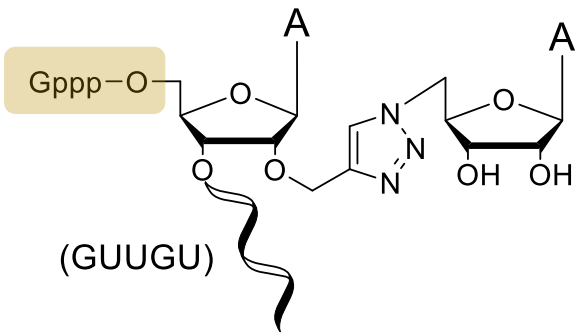
Inhibition of NS5 Dengue (DV) / Zika (ZK)

❑ *With / without cap structure*

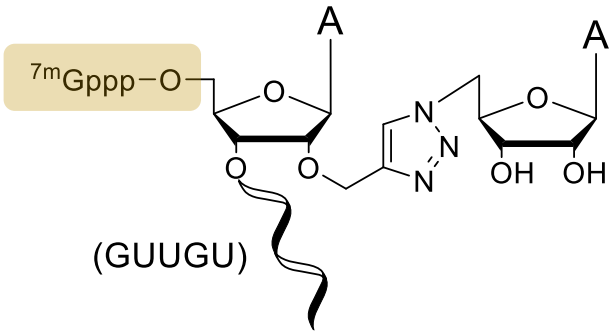
Cap structure is crucial !



DV : NI at 10 µM

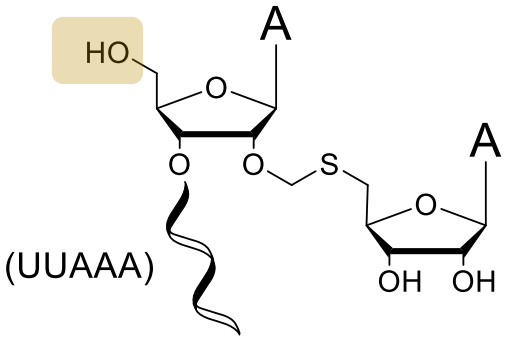


DV : IC_{50} = 169 nM
ZK : IC_{50} = 237 nM

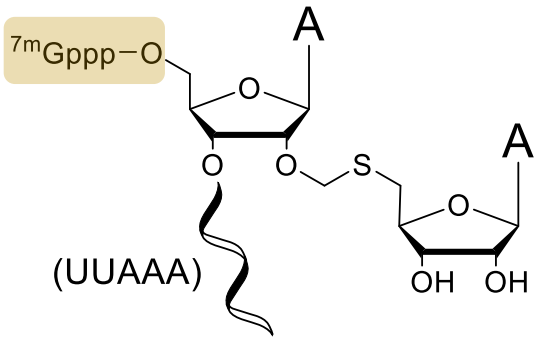


DV : IC_{50} = 230 nM

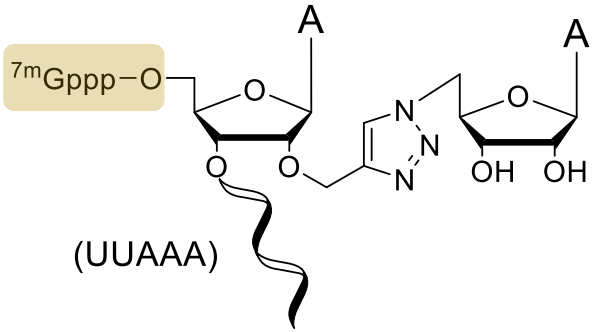
Inhibition of SARS-CoV-2 nsp10/nsp16



CoV-2 : NI at 10 µM



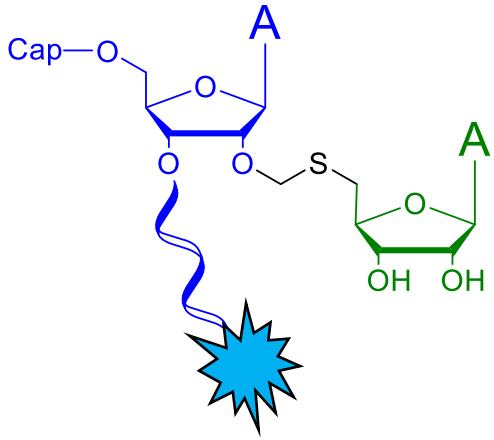
CoV-2 : IC_{50} = 511 nM

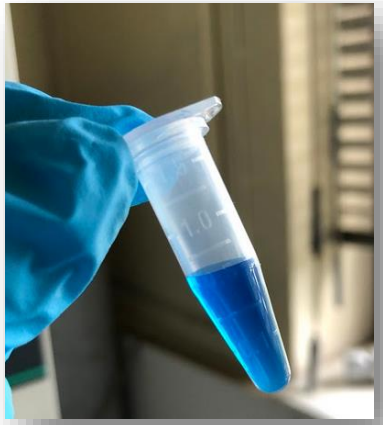


CoV-2 : IC_{50} = 172 nM

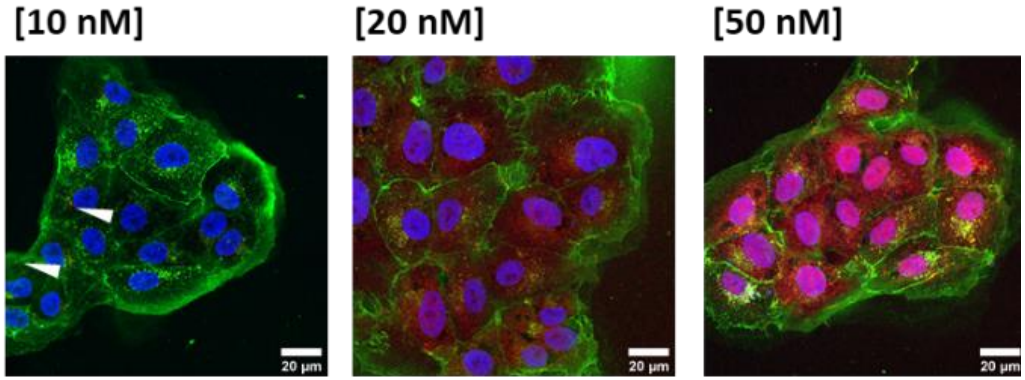
Perspectives – Cellular Inhibition Assays

1) Preparation of Cy5-labelled oligonucleotides (Synthesis not shown for today)





2) Cellular internalization of encapsulated Cy5-oligonucleotides (peptides)



Represented images of VERO cells incubated with Cy5-Oligos

Oligo/peptide nanoparticles enter the cell ! Measurement of SARS-CoV-2 viral infection in infected cells ongoing

Lab "PhyMedExp" - Montpellier
Dr. Karidia Konaté, Dr. Prisca Boisguérin

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Dr. Françoise Debart

Dr. Jean-Jacques Vasseur

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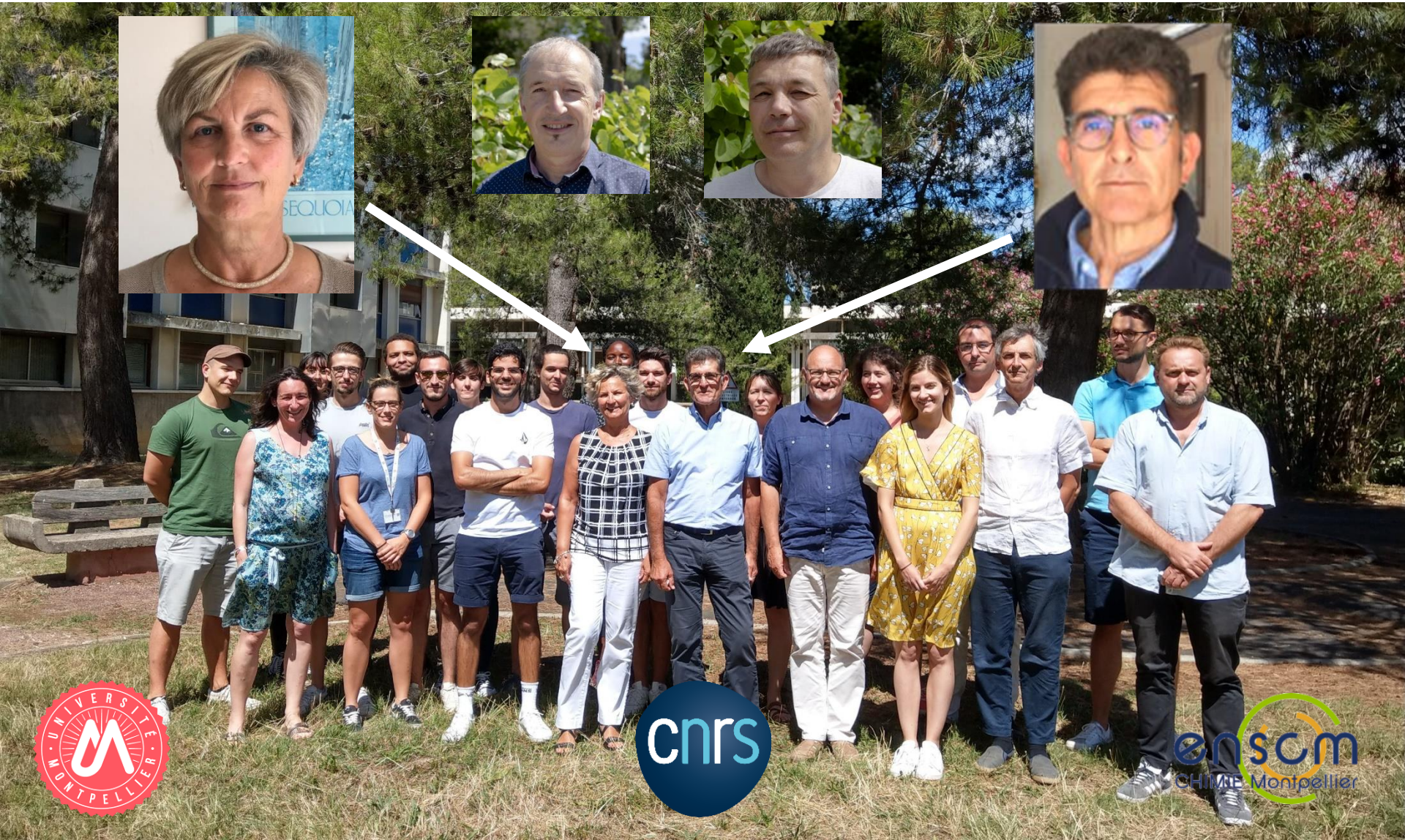
Dr. Etienne Decroly



Acknowledgments

Marcel Hausdorff Joris Troussier Mathieu Noel