Fragment-based Drug Discovery of Pan-Coronavirus Antivirals Assisted by Generative Machine Learning:

Targeting the SARS-CoV-2 Helicase (nsp13)

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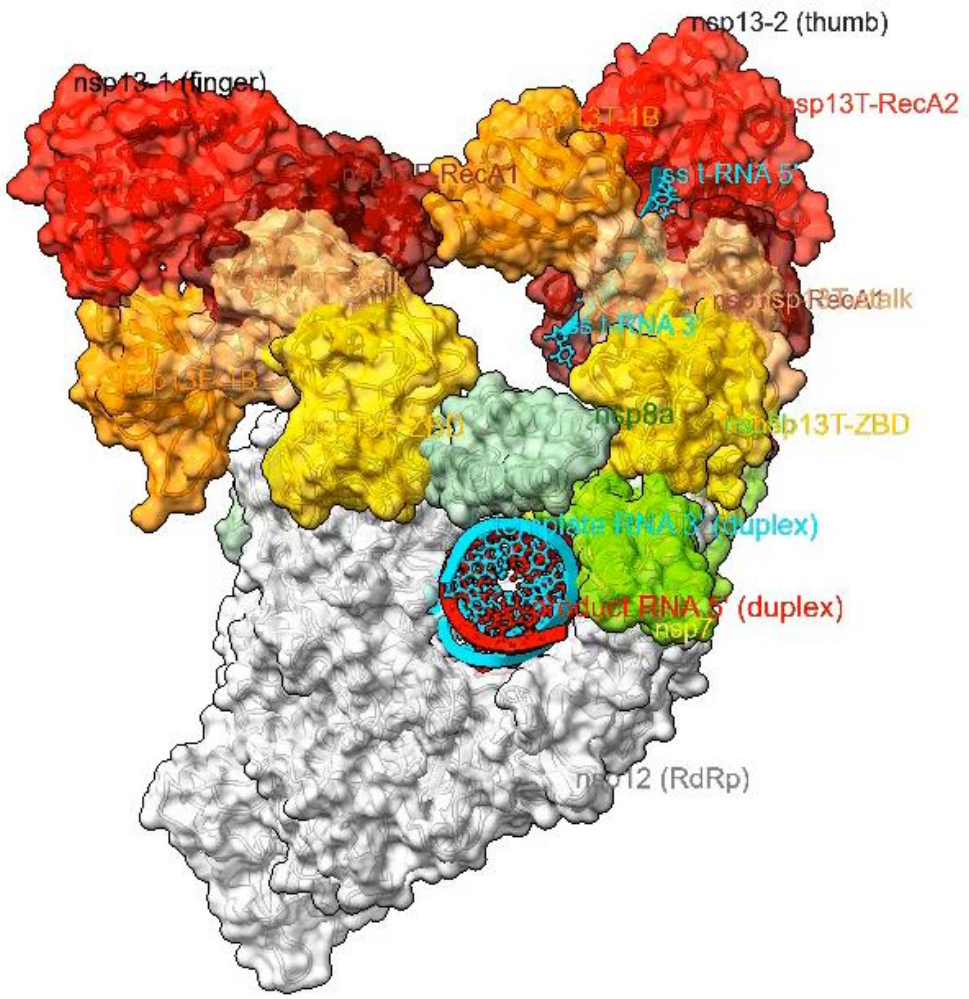
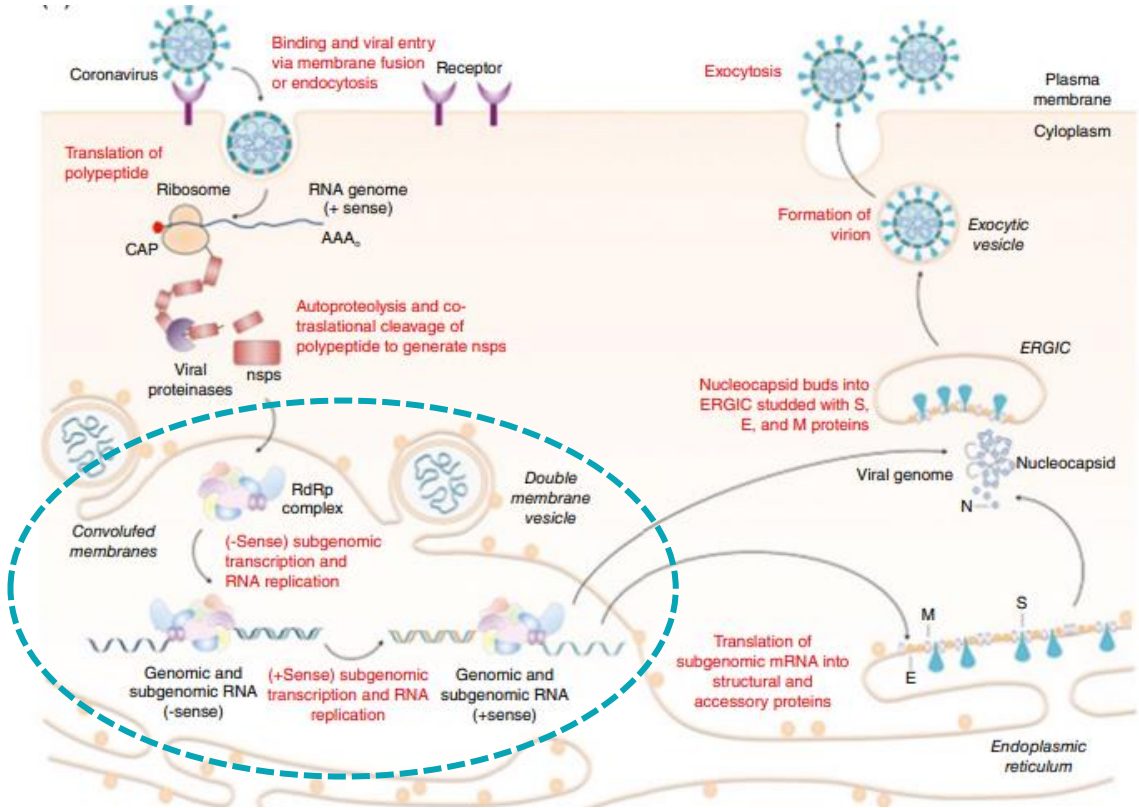
UCL School of Pharmacy

SARS-CoV-2 Replication Transcription Complex (RTC):
The (+)ssRNA replicative machinery of the coronavirus (PDB 7CXN)

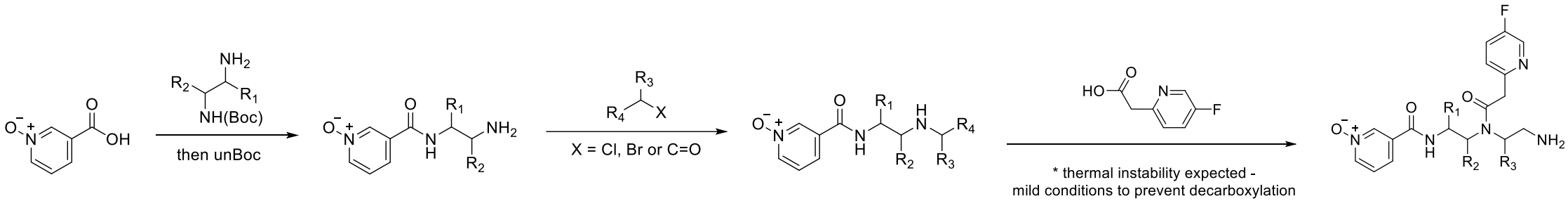


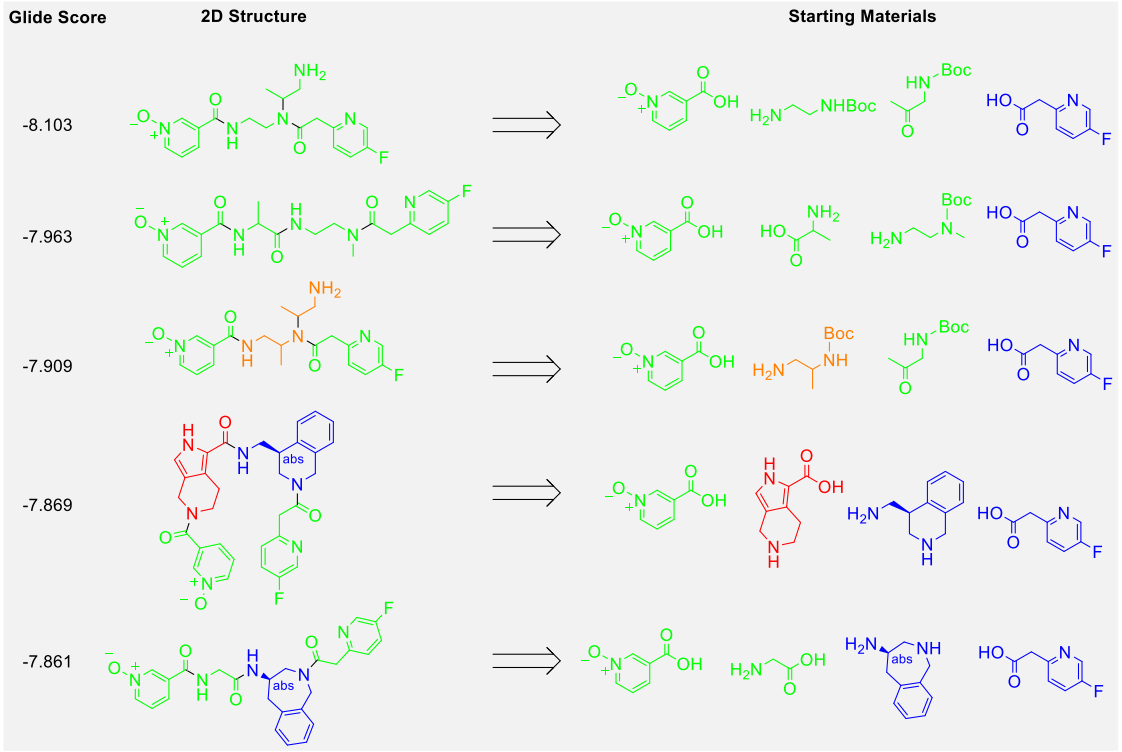


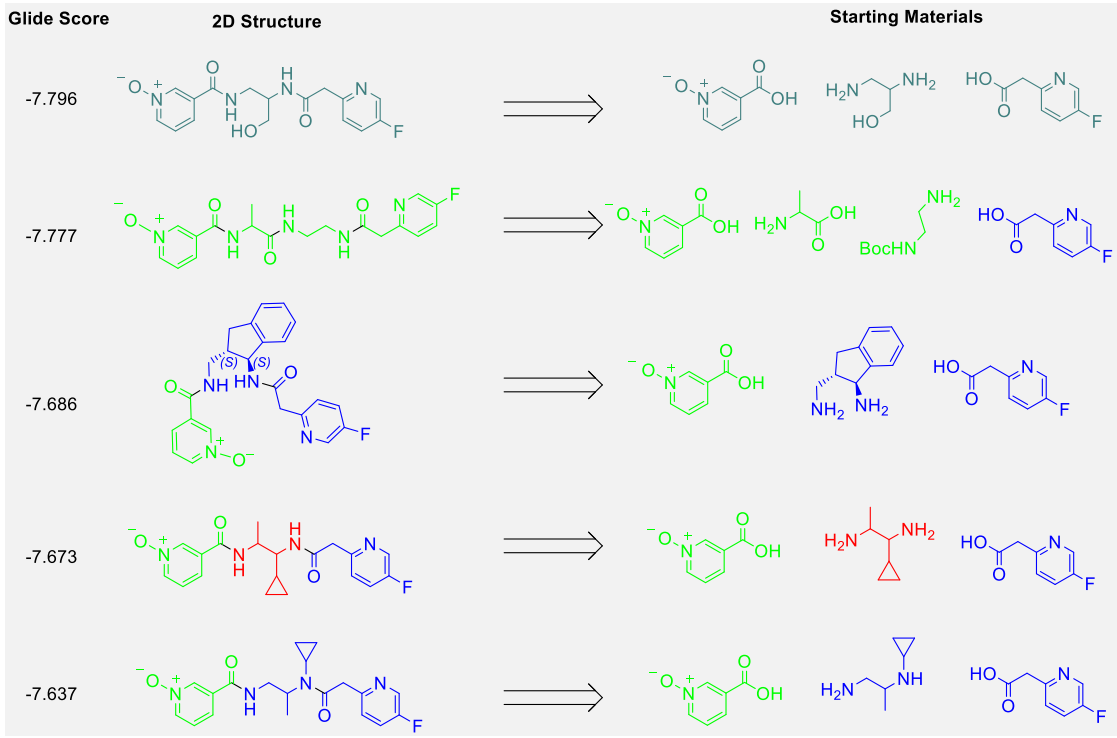
Chen, J., Wang, Q., Malone, B. *et al.* Ensemble cryo-EM reveals conformational states of the nsp13 helicase in the SARS-CoV-2 helicase replication–transcription complex. *Nat Struct Mol Biol* **29,** 250–260 (2022). https://doi.org/10.1038/s41594-022-00734-6

Generated 100 List Library Planning:
Cluster around -8.103

Cheap acid starting material and amine building blocks can be used for amide synthesis…

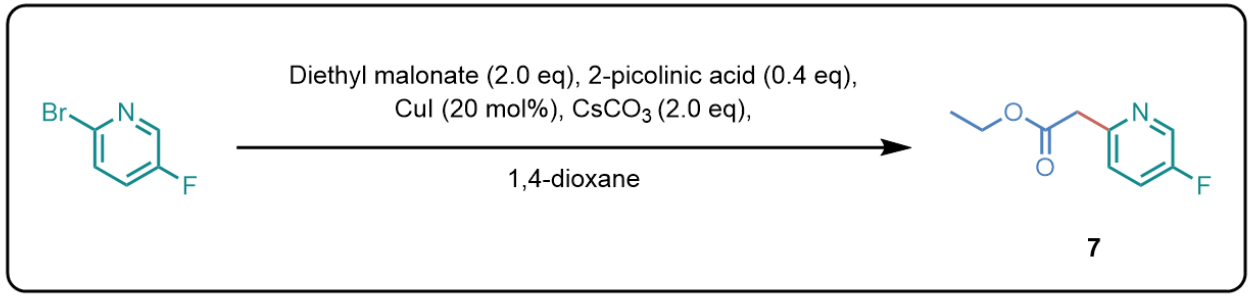


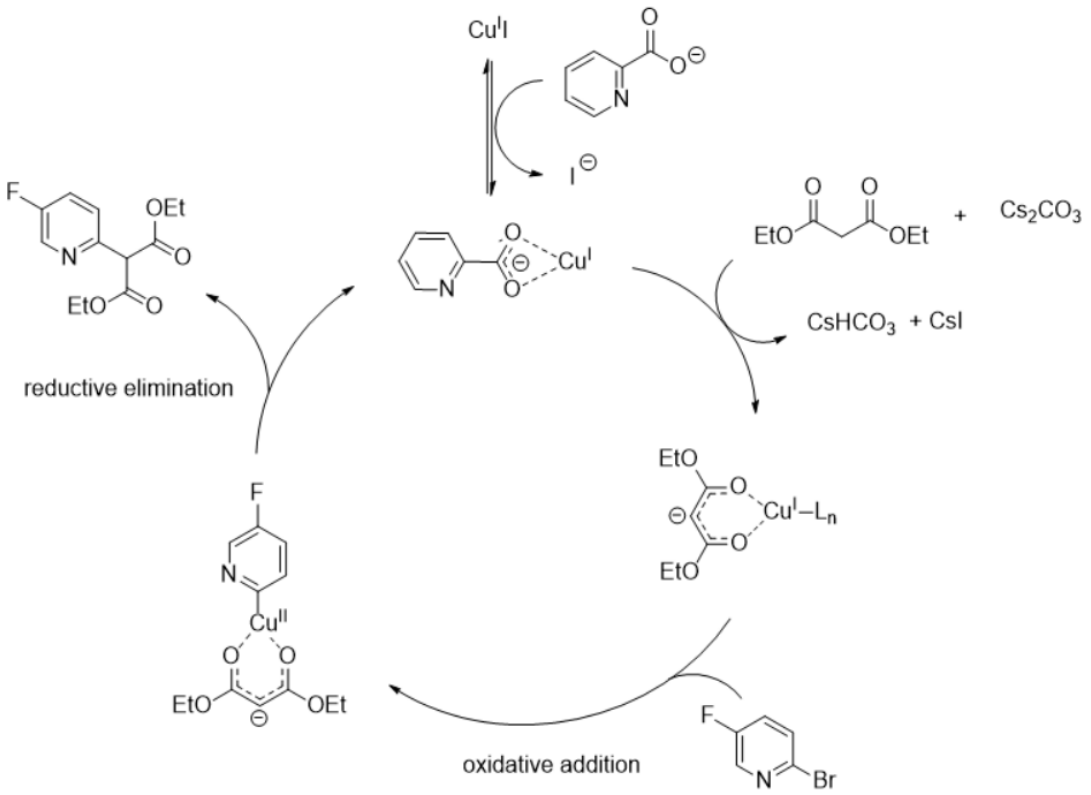




- Purchased
- To Buy
- Make
- Unavailable
- Enamine MOD

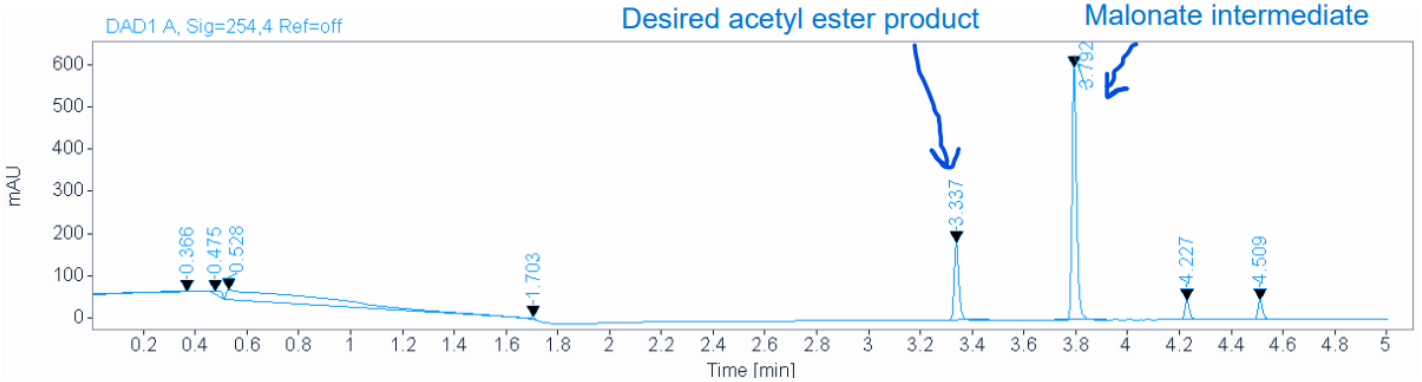
TK-41-3: Preparation of ethyl 2-(5-fluoropyridin-2-yl)acetate. - Ullman-type coupling





- 3 routes attempted – 1 successful.
- Highly air and moisture sensitive = difficult scale-up.
- Currently have 328 mg of ~1:3 mixture of ethyl ester vs malonate intermediate (based on a theoretical yield of 522 mg); see next slide.
- Attempted to separate, but failed despite multiple attempts.
- Plan to decarboxylate the remaining intermediate in hopes of isolating pure ethyl ester product.

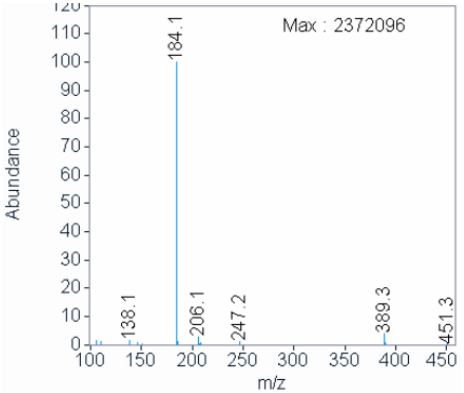
TK-41-3: Preparation of ethyl 2-(5-fluoropyridin-2-yl)acetate. - Ullman-type coupling



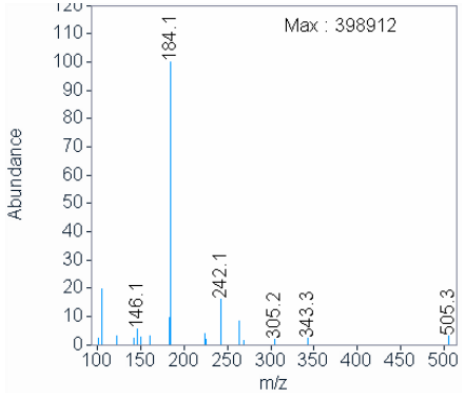
Signal: DAD1 A, Sig=254,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Nam
0.366	BB	0.2583	35.0443	1.6043	1.8375	
0.475	BB	0.0458	25.5569	7.3055	1.3400	
0.528	BB	0.3856	755.4314	23.3088	39.6089	
1.703	BB	0.0136	5.4094	6.2657	0.2836	
3.337	BB	0.0193	233.2522	183.8747	12.2299	
3.792	BB	0.0190	740.9135	597.6688	38.8477	
4.227	BB	0.0186	55.2647	45.7995	2.8977	
4.509	BB	0.0192	56.3520	46.1463	2.9547	
		Sum	1907.2244			

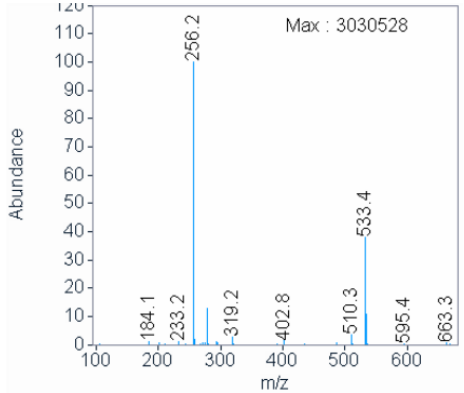
Peak RT 3.357



Peak RT 3.591



Peak RT 3.822

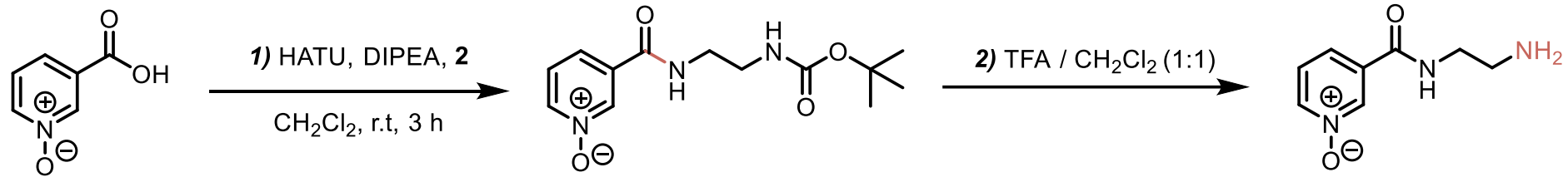


328 mg, yellow oil.

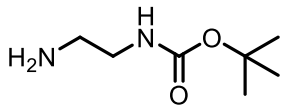
Theoretical 522 mg, 2.85 mmol)

A separation method needs to be developed.

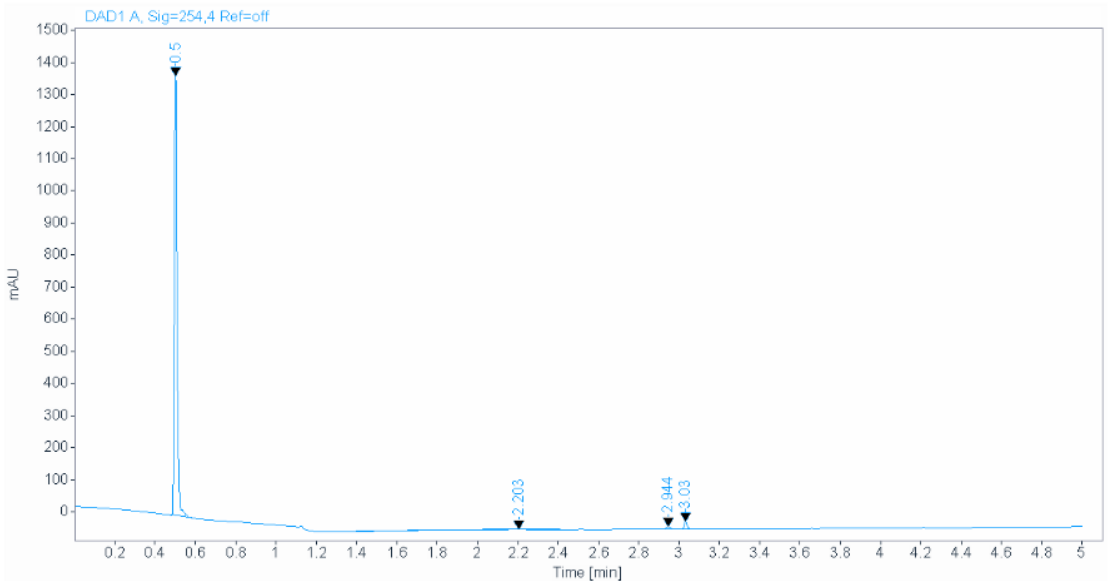
TK-42-3 / -43-2: Amidation and de-Boc of amide using TFA (2.5 mmol scale).



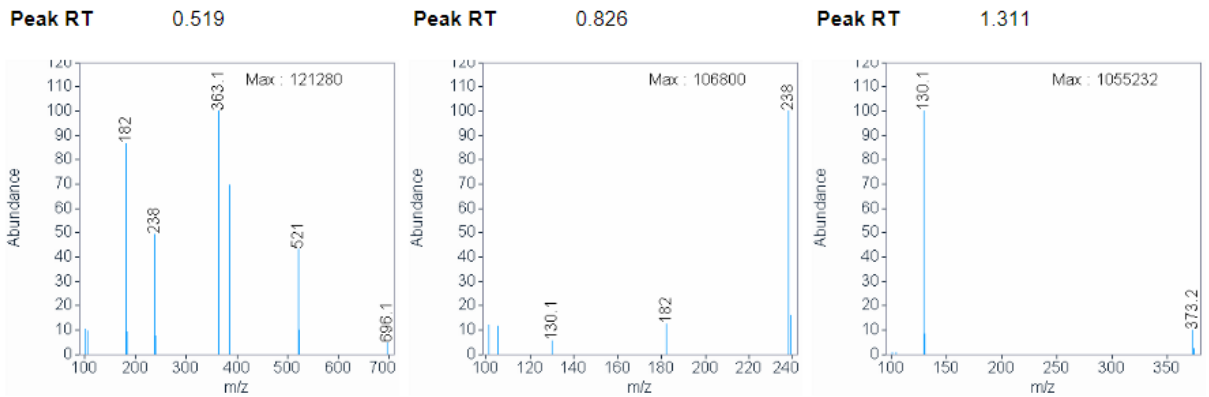
1 **2** **3** **4**



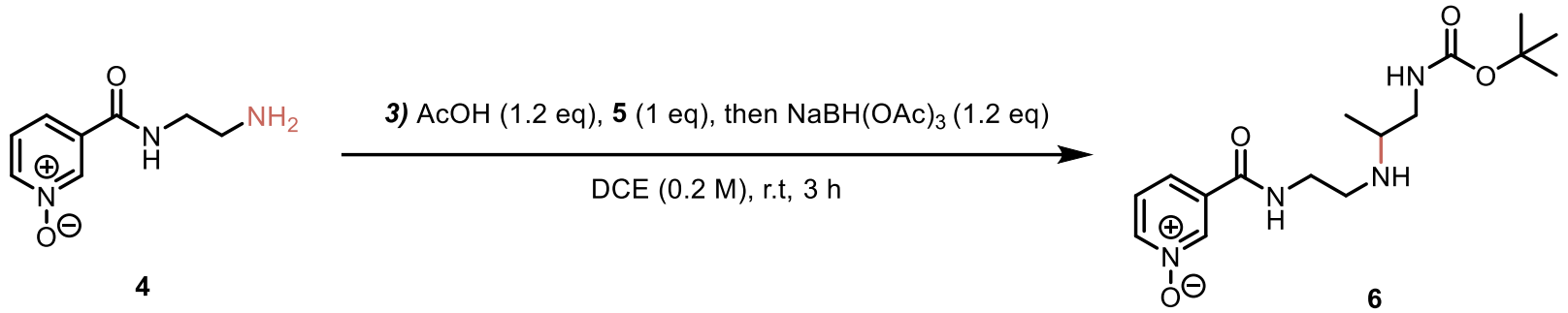
tert-butyl (2-aminoethyl)carbamate

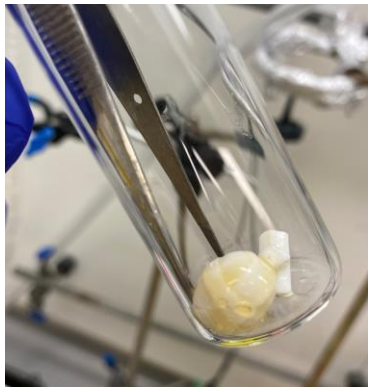


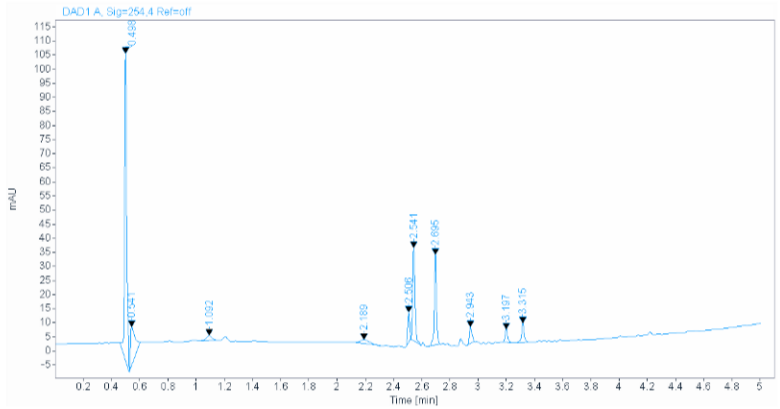
- Successful isolation of primary amine intermediate based on the route shown above (based on 1.5 mmol scale).
- Purification of **3** means that purification of the primary amine **4** is not required.



TK-44-2: Reductive amination with NaBH(OAc)3 (1.50 mmol scale).

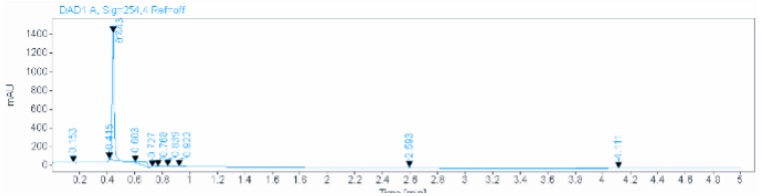






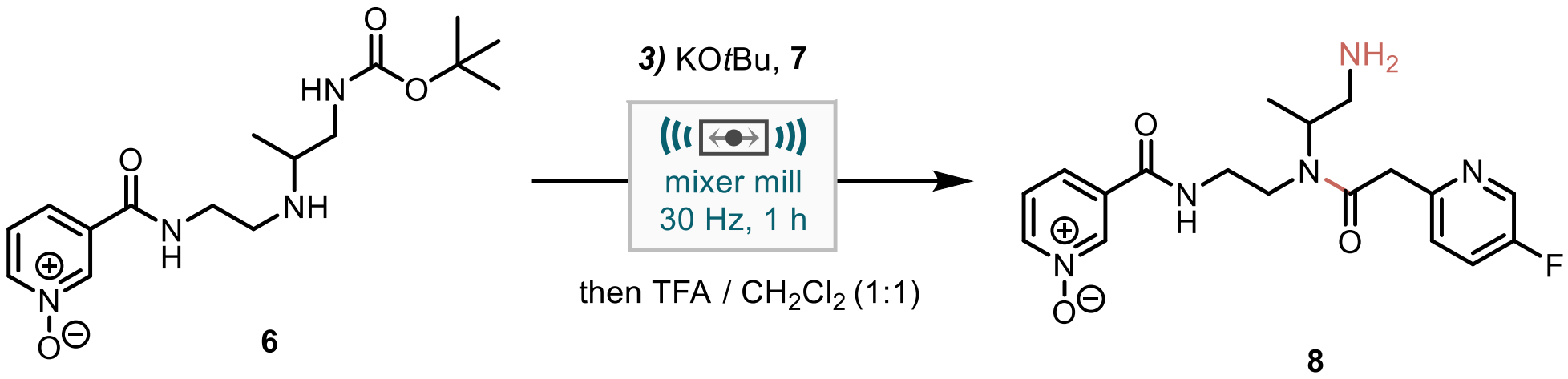
Data file: C:\Users\Public\Documents\ChemStation\1\Data\TODD\TK\TK 2022-10-07 15-02-43\001-P1-B2-TK-44-1-B-aqeous.D
Sample name: TK-44-1-B-aqeous
Description:
Sample amount: 0.000
Sample type: Sample

Instrument: G25LCMS
Injection date: 10/7/2022 3:04:48 PM
Acq. method: EGT_5min pos.M
Analysis method: EGT_5min pos.M
Last changed: 7/27/2022 1:12:31 PM
Location: P1-B2
Injection: 1 of 1
Injection volume: 1.000
Acq. operator: Tom Knight



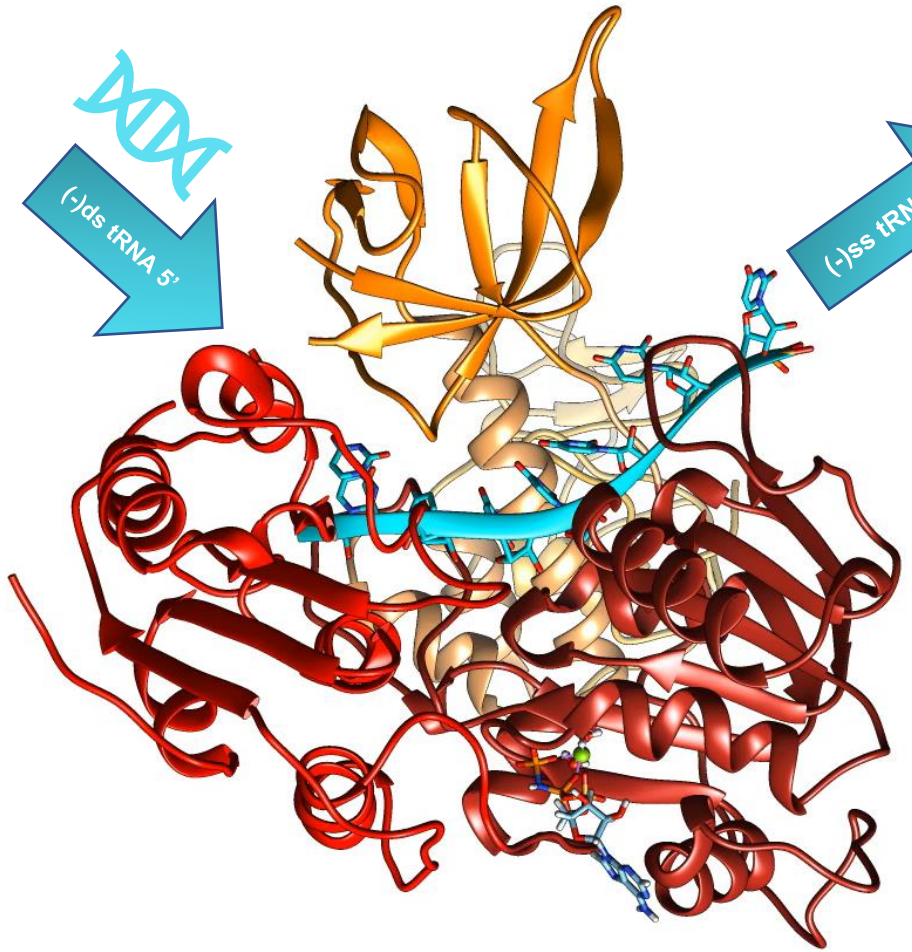
- Difficulty with reductive amination step – likely due to failure to form an imine intermediate from ketone.
- May be easier to form imine in one step, then reduction in the subsequent step (i.e. not one-pot).

Planned: Direct amidation from the unstable ethyl ester using mechanochemistry



- Assuming successful, will attempt direct ball-milled amidation of the secondary amine from the ethyl ester to prevent decomposition of the unstable 5-fluoropyridine carboxylic acid (prone to decarboxylation).

Nsp13 Helicase (+)ssRNA Translocation Mechanism:
Based on H/D exchange assays, X-ray Diffraction



- 7NIO apo-nsp13
- 7NNO nsp13-ANP
- 6JYT nsp13
- 2XZL Upf1-RNA

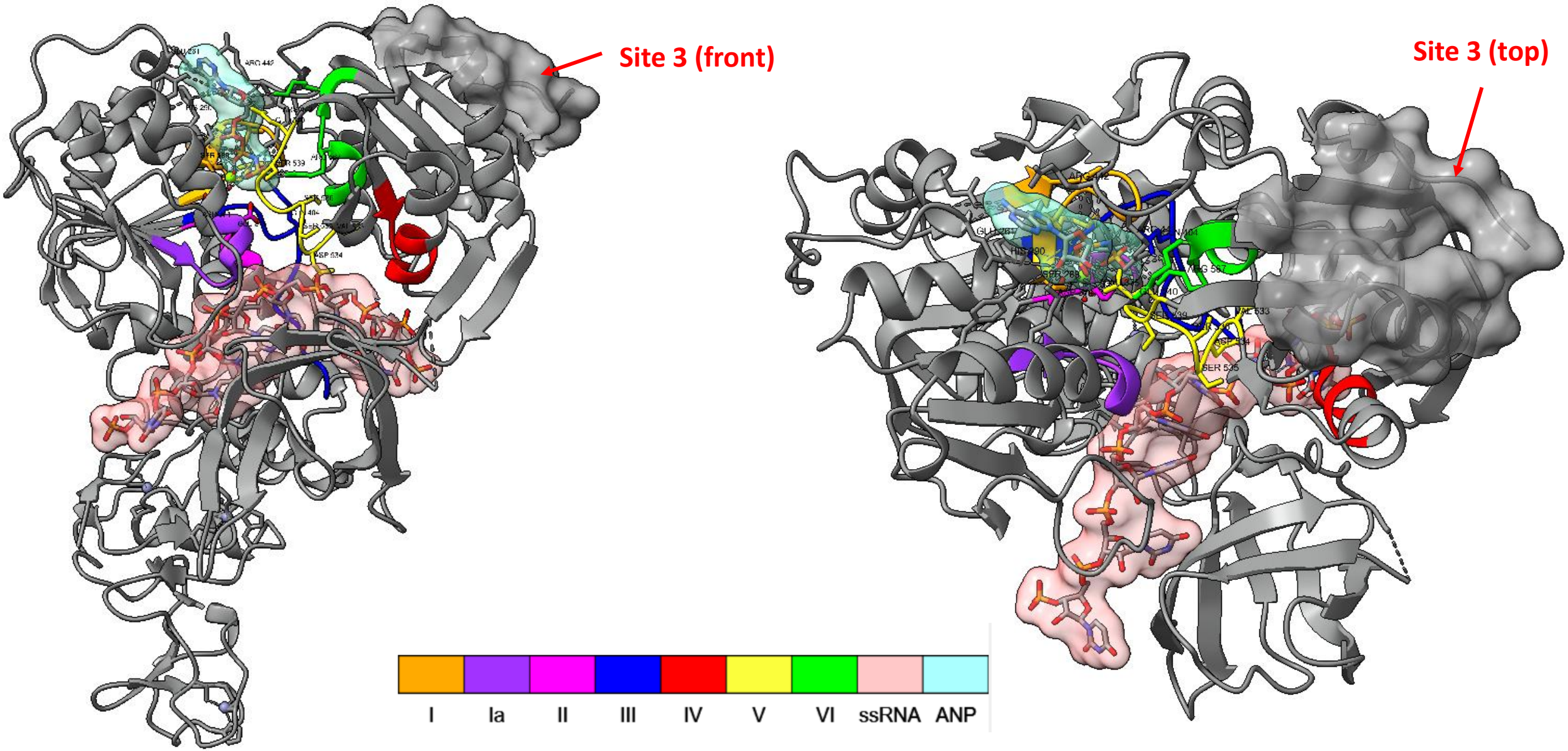
- Rec 1A domain
- Rec 2A domain
- 1B domain
- Stalk domain
- Zinc-binding domain
- AMP-PNP (ANP)
- (+)ssRNA strand

Enzyme activity:

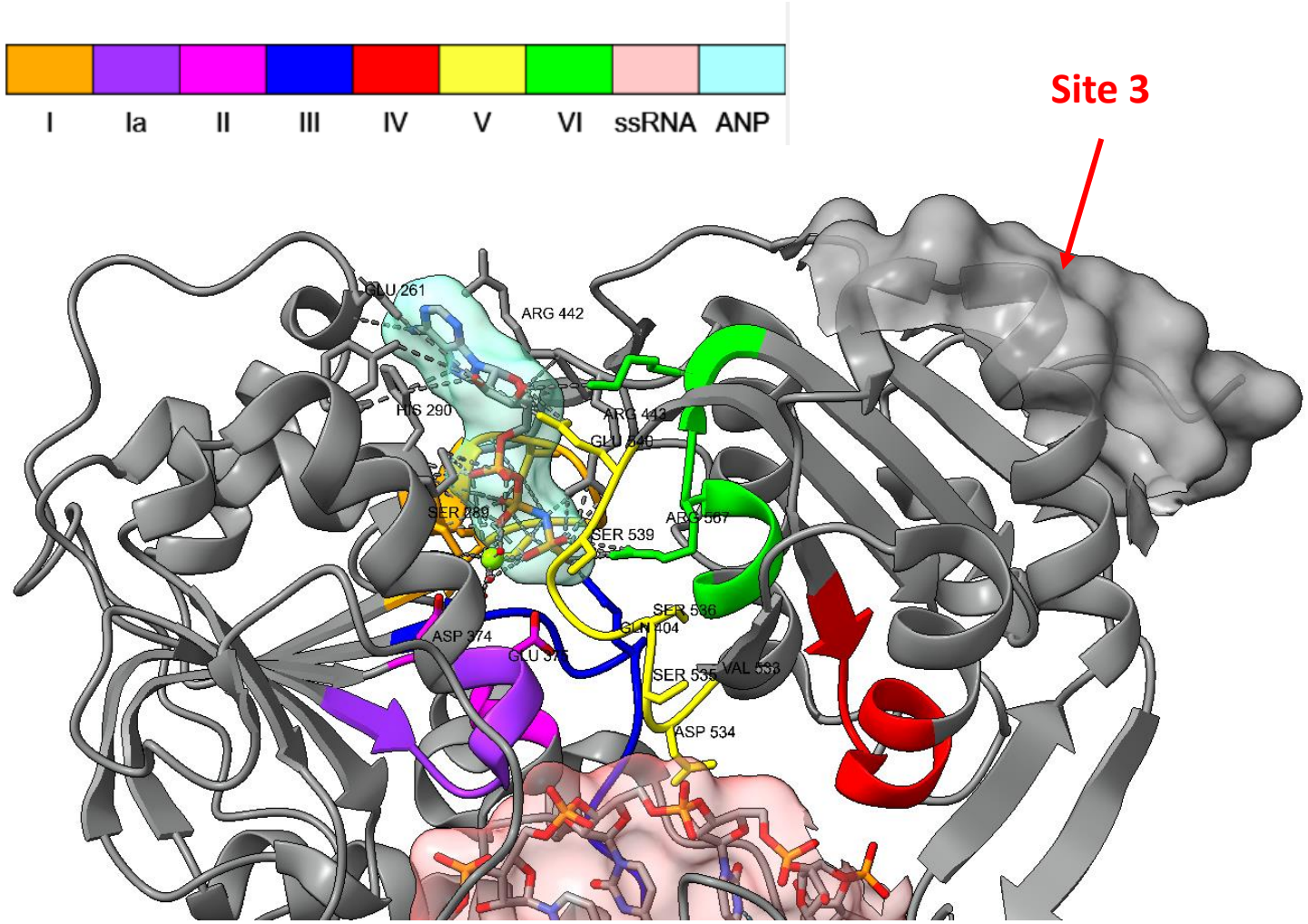
- ATP hydrolysis (ATPase), RNA unwinding (helicase).
- Helicase is activated by binding to ATP and a 3′ ssRNA overhang.
- RNA is threaded through a channel between the RecA domains.
- Unwinding of RNA duplex is coupled to ATP hydrolysis and processive translocation of the helicase by one nucleotide.
- A **four-step inchworm stepping translocation mechanism of nsp13 along ssRNA** has been proposed, motifs Ia and IV act as binding sites that independently bind and release the phosphates of ssRNA (see next slide).

1. Zhihui Jia et al.Nucleic Acids Research, 2019, Vol. 47, No. 12, 6538–6550 doi: 10.1093/nar/gkz409 **2.** Newman, J.A. *et al.* Structure, mechanism and crystallographic fragment screening of the SARS-CoV-2 NSP13 helicase. *Nat Commun* **12,** 4848 (2021). https://doi.org/10.1038/s41467-021-25166-6. PDB 7NIO (apo-nsp13), 7NN0 (ANP-bound), 6JYT (ATP hydrolysis), 2XZL (Upf1 helicase / ssRNA)

Nsp13: Site 3 relative to ATP and RNA binding motifs



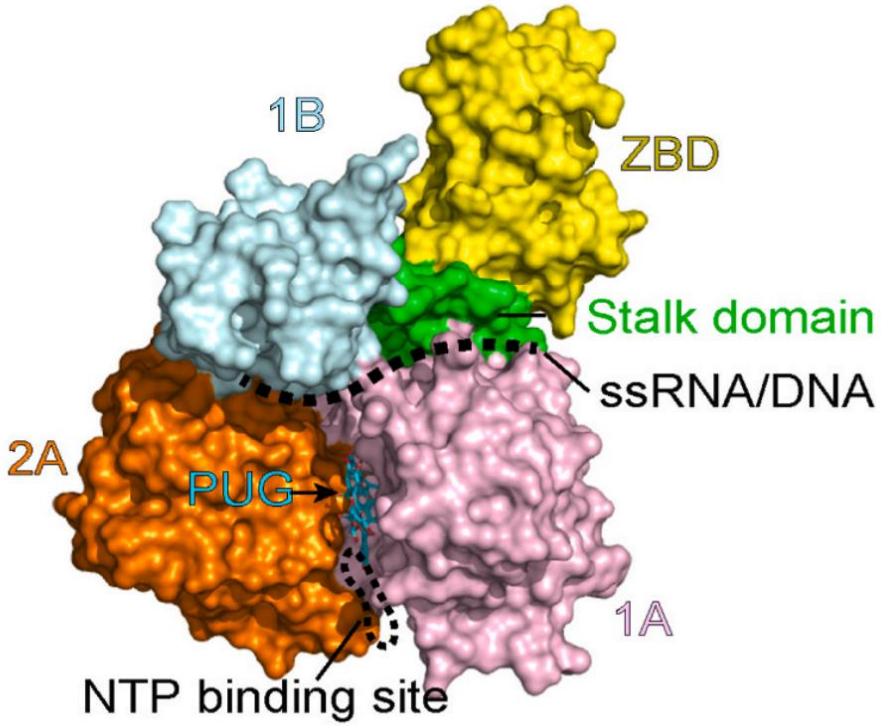
Nsp13: Site 3 relative to ATP and RNA binding motifs

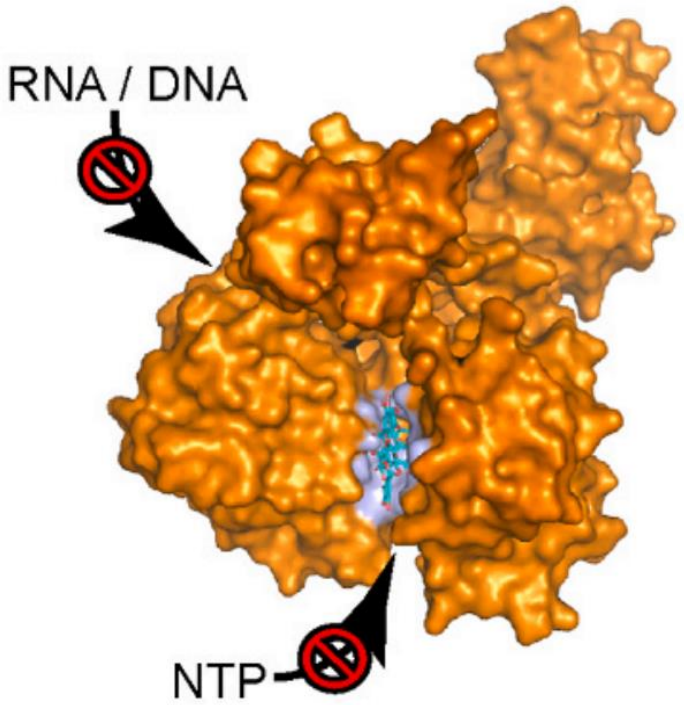


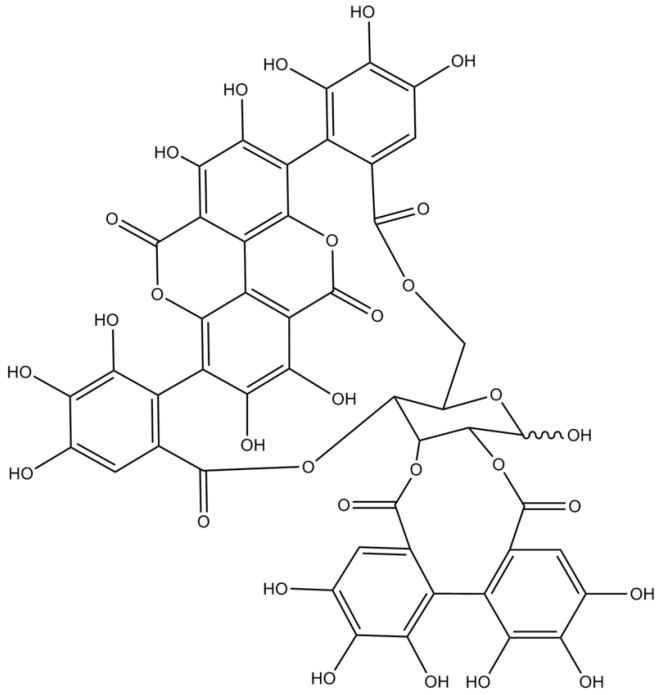
- **Nucleoside Triphosphate (ATP) Binding and Hydrolysis Motifs:** Motifs **I** & **II**
- **RNA / DNA Binding & Unwinding Motifs:** Motifs **Ia** & **IV**
- **Motifs connecting the two binding regions:** motifs **III**, **V**, **VI**).
- Molecular dynamics simulations of the flavivirus NS3 helicase protein revealed motif **V** as an **allosteric link between the ATP-binding pocket and the RNA-binding cleft** due to strong correlations between motif **V** and both binding pockets.
- These results were supported by mutagenesis studies both in vitro and in silico.
- SARS-CoV-2 **motif V** and subdomain **Rec2A** are more dynamic than their flaviviral homologues, but the mechanistic role of motif V is conserved.

Source: Role of ATP in the RNA Translocation Mechanism of SARS-CoV-2 NSP13 Helicase *J. Phys. Chem. B* 2021, 125, 31, 8787-8796. DOI: 10.1021/acs.jpcb.1c04528 https://doi.org/10.1021/acs.jpcb.1c04528

PUG: A nanomolar allosteric Nsp13 inhibitor (SPR assay)







Punicalagin (PUG)

- IC50 : 427 nm
- KD : 21.6 nm
- SPR based on amine coupling to a Cytive CM5 chip using His-tagged Nsp13.

L. Lu et al. Antiviral Research 206 (2022) 105389. https://doi.org/10.1016/j.antiviral.2022.105389