

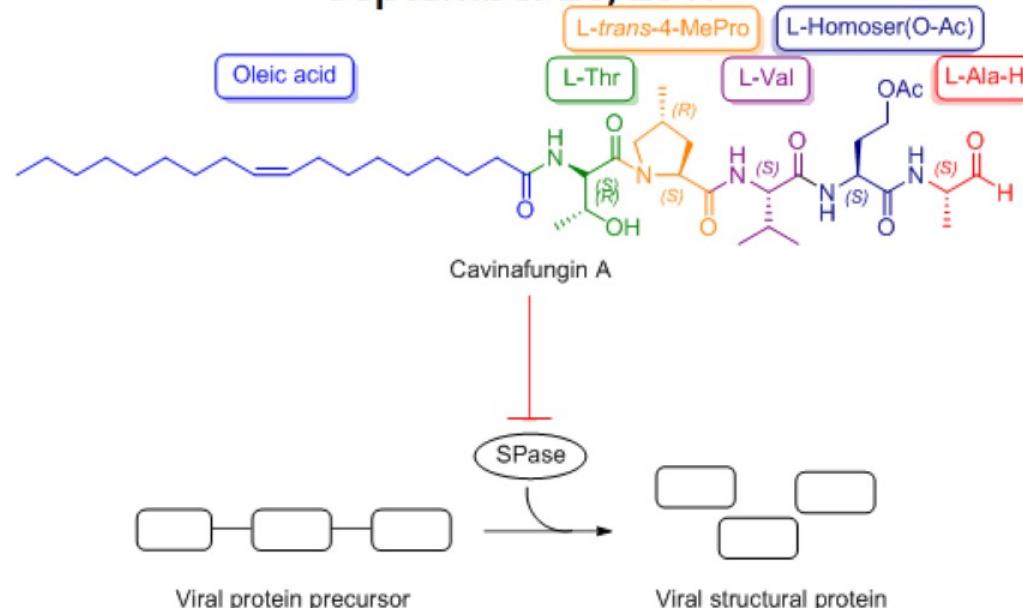


Synthesis and Structural Modifications of Antiviral Natural Product Cavinafungin A

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September 20, 2017



Zika and dengue affected regions

- Dengue infection annual average – 925,896 (00-04)
- 2015-17 Total Zika cases – ~1 billion

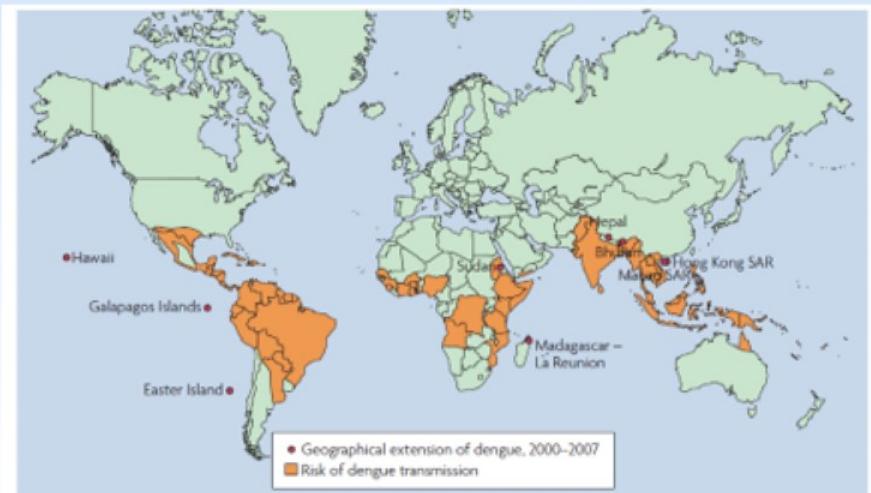
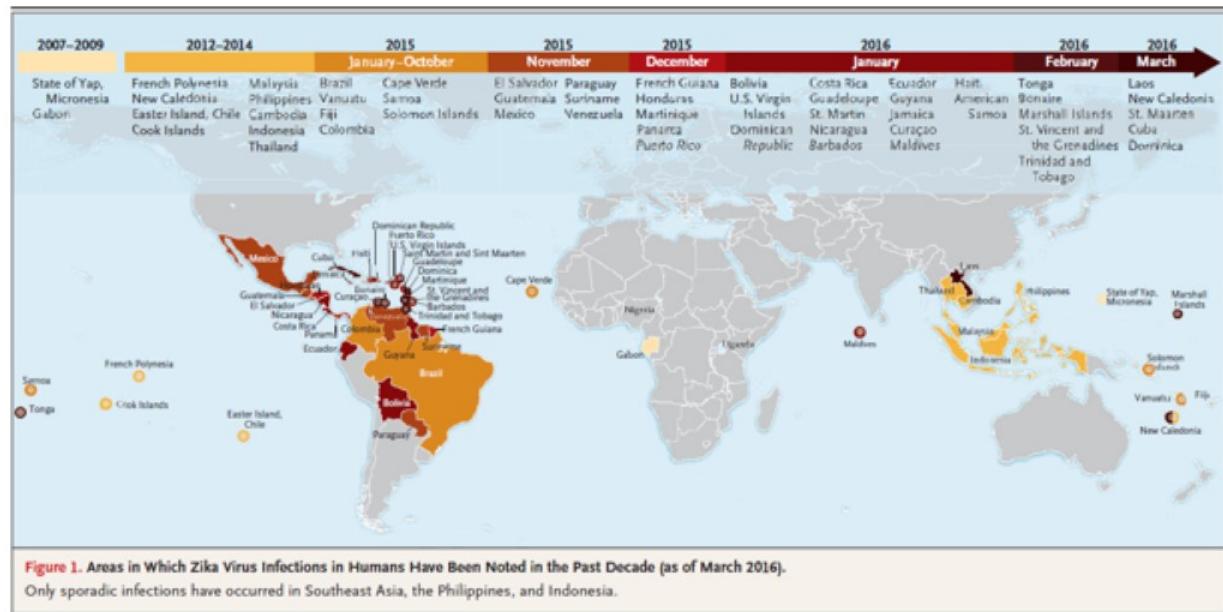
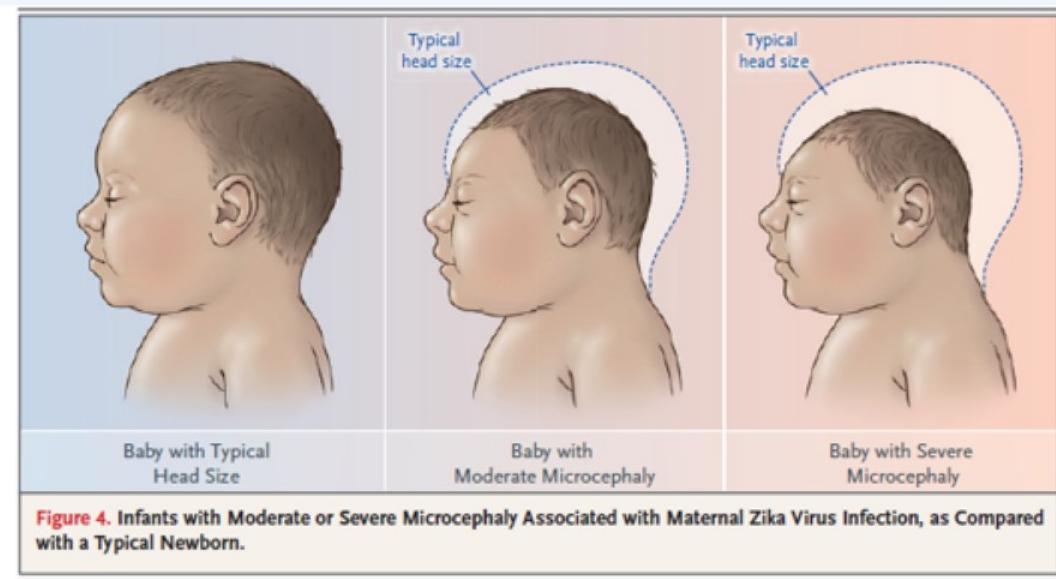
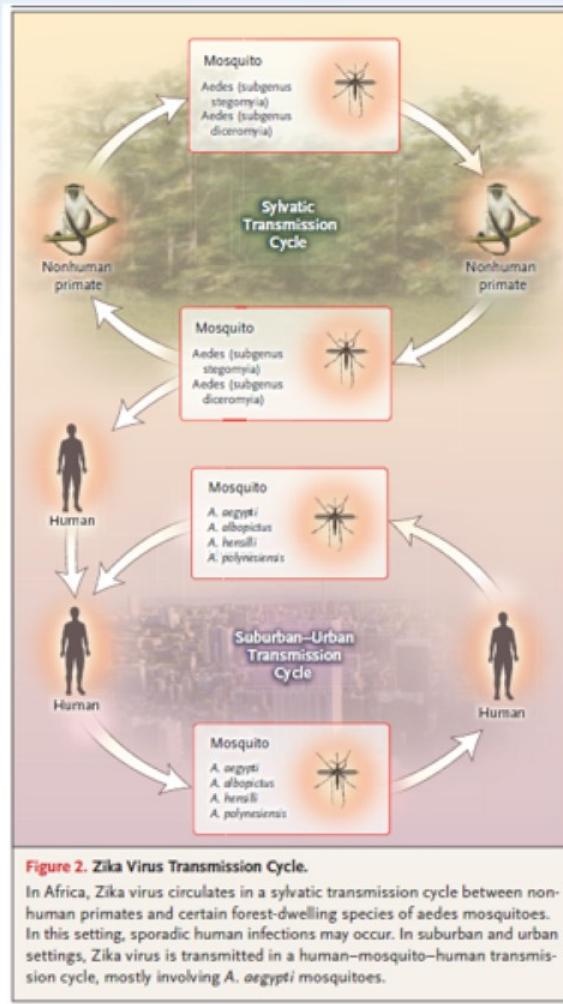


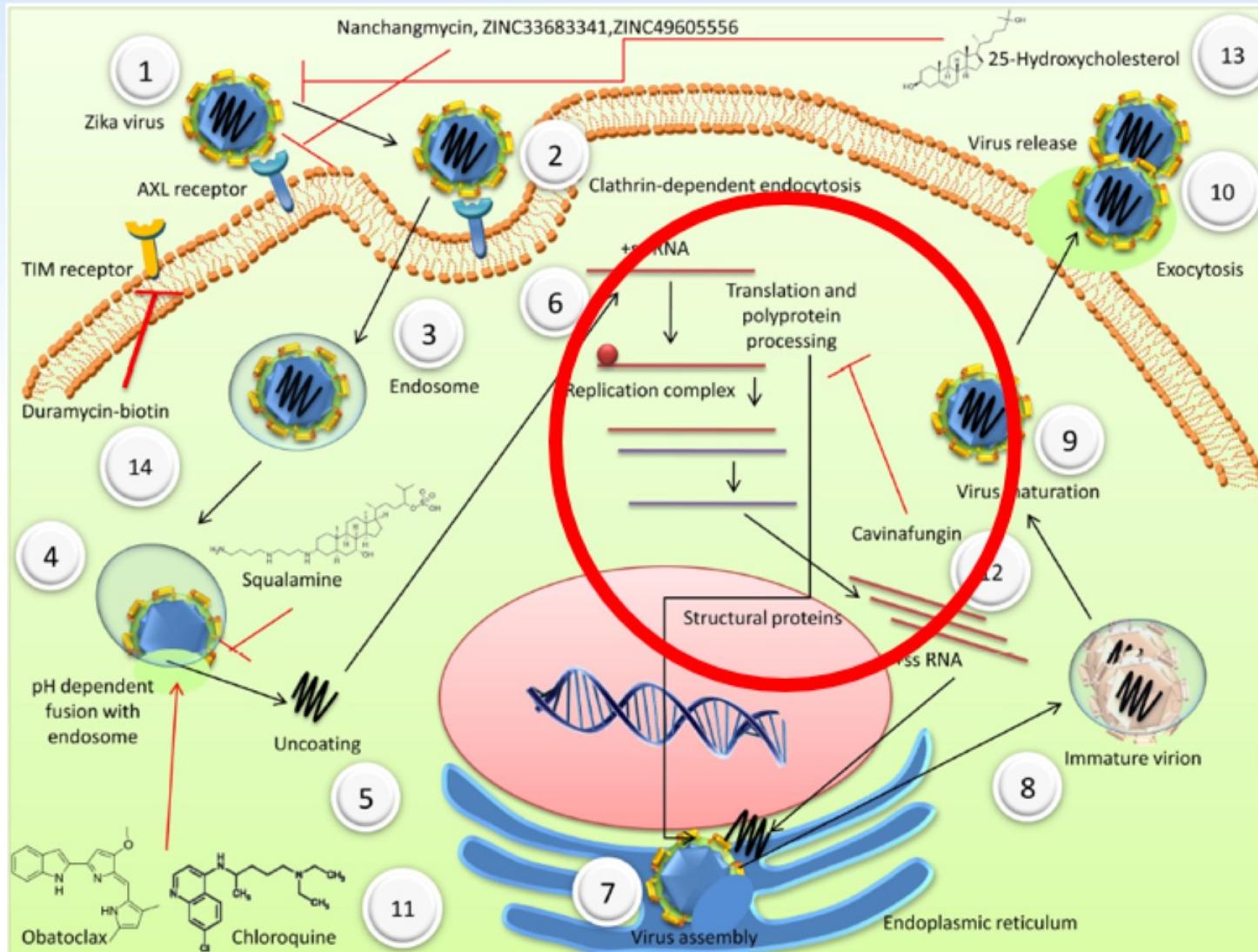
Figure 1 | Countries and areas at risk of dengue transmission, 2007. Data from WHO.



- No specific treatments for Zika/dengue infections

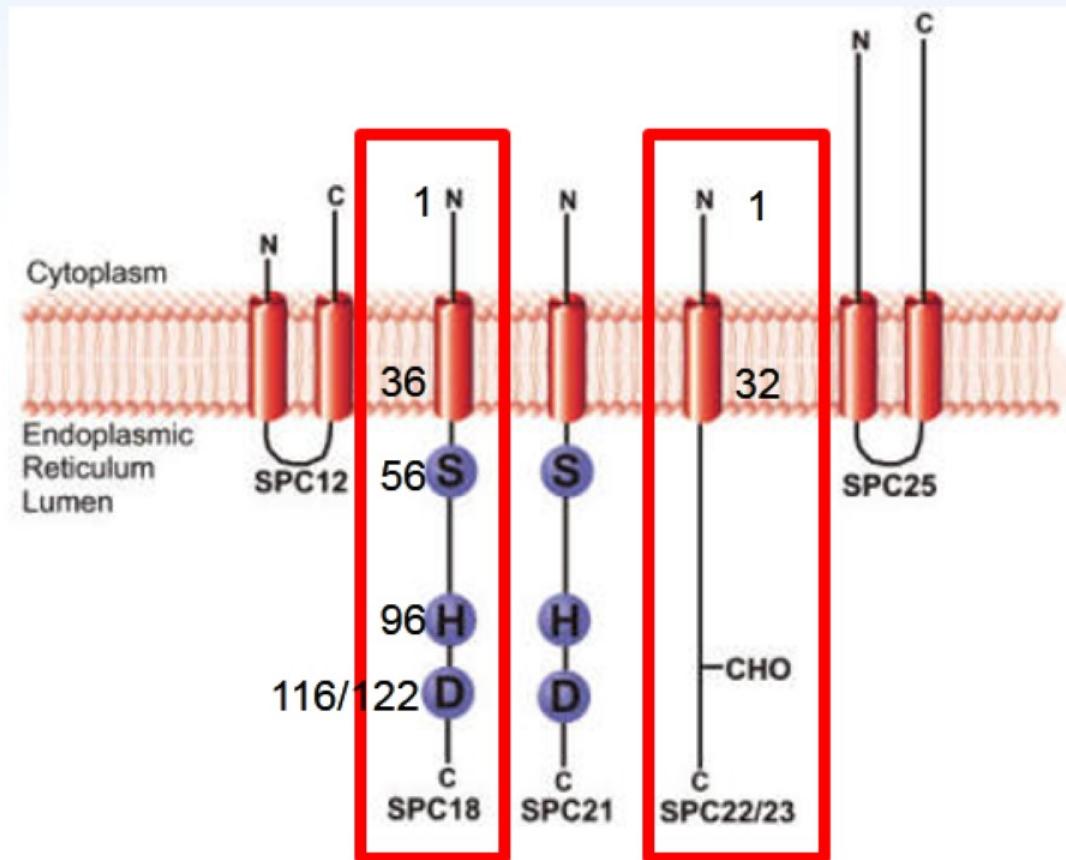


Zika viral infection mechanisms



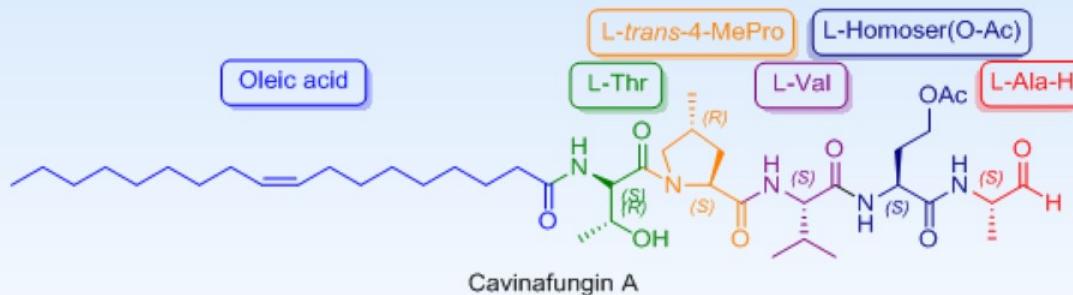
Human SPase complex

- SPC18 (aka SEC11A) – Catalytic domain, direct CavA target
- SPCS3 (aka SPC22/23) – Essential protein coenzyme



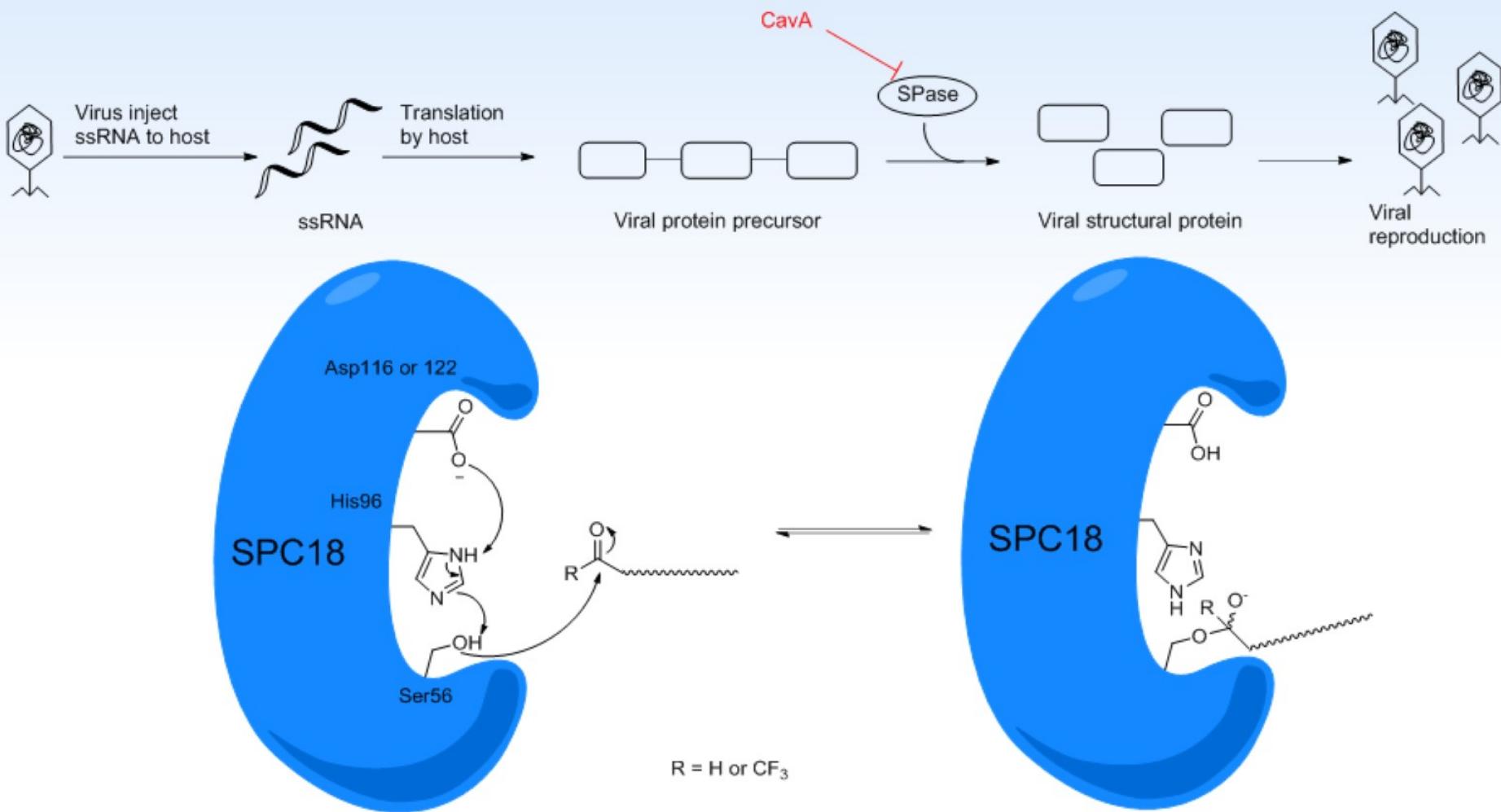
Paetzel, M., et al., *Chemical Reviews* 2002, 102(12), 4549-4580.
Shelness, G. S., et al., *Journal of Biological Chemistry* 1993, 268(7), 5201-5208.

Antiviral activities of Cavinafungin A (CavA)



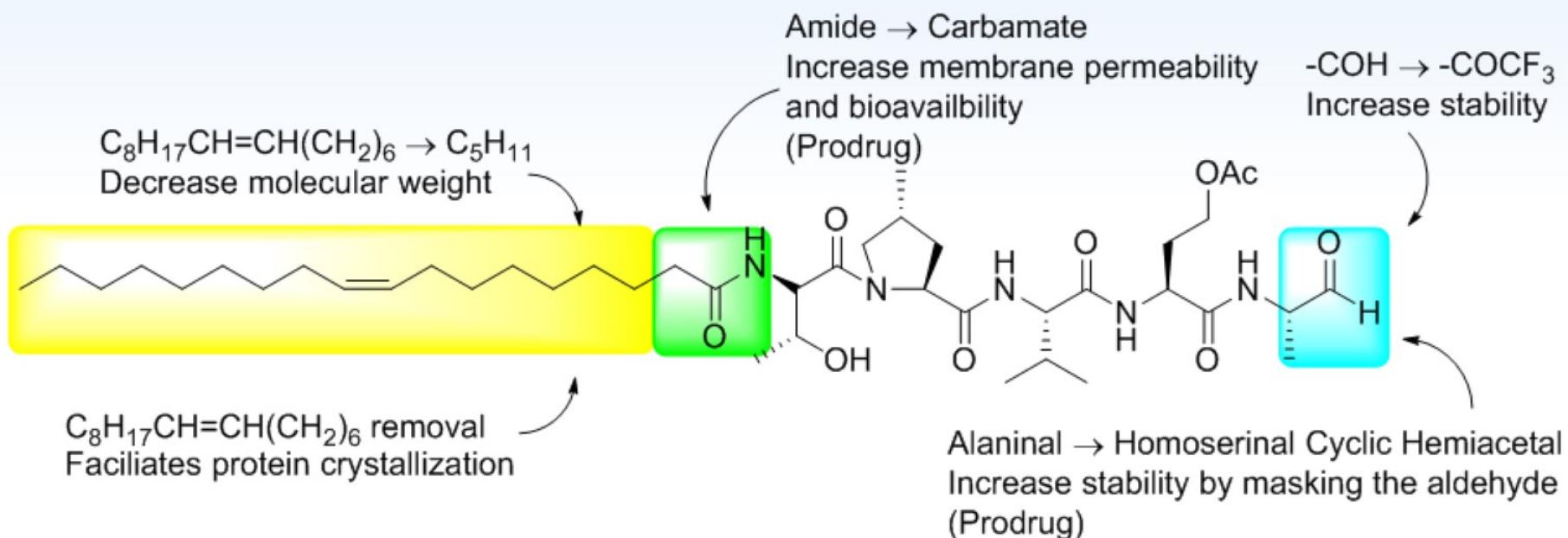
Virus	Cell Line	IC ₅₀ / CV (nM) / (%)	CC ₅₀ / CV (nM) / (%)
Cavinafungin	DV1	4 / 57	800 / 36
	DV2	5 / 71	800 / 36
	DV2*	4 / 49	2'000 / n.d.
	DV2°	1 / n.d.	18'000 / n.d.
	DV3	3 / 22	800 / 36
	DV4	3 / 27	800 / 36
	ZV	150 / 23	1'650 / 49
	CHIKV	900 / 24	2'000 / n.d.

Antiviral mechanism



Structural modification summary

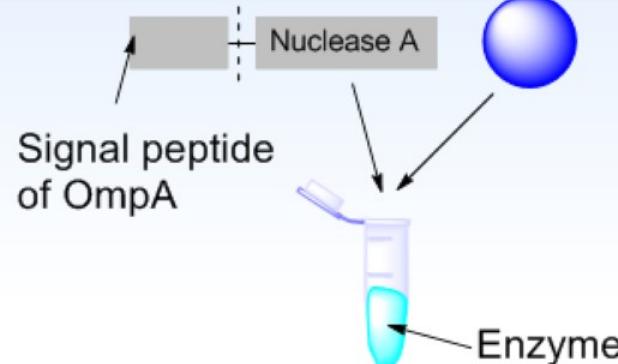
- Improve chemical and pharmacological properties



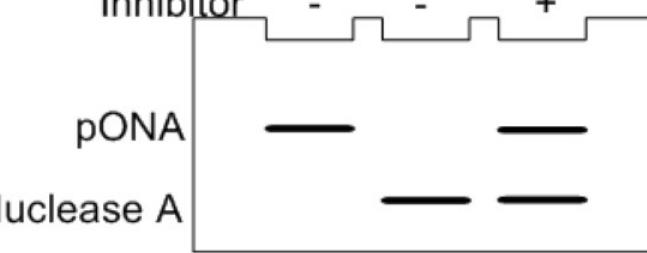
In vitro activity assay

In vitro substrate
pro-OmpA-Nuclease A (pONA)

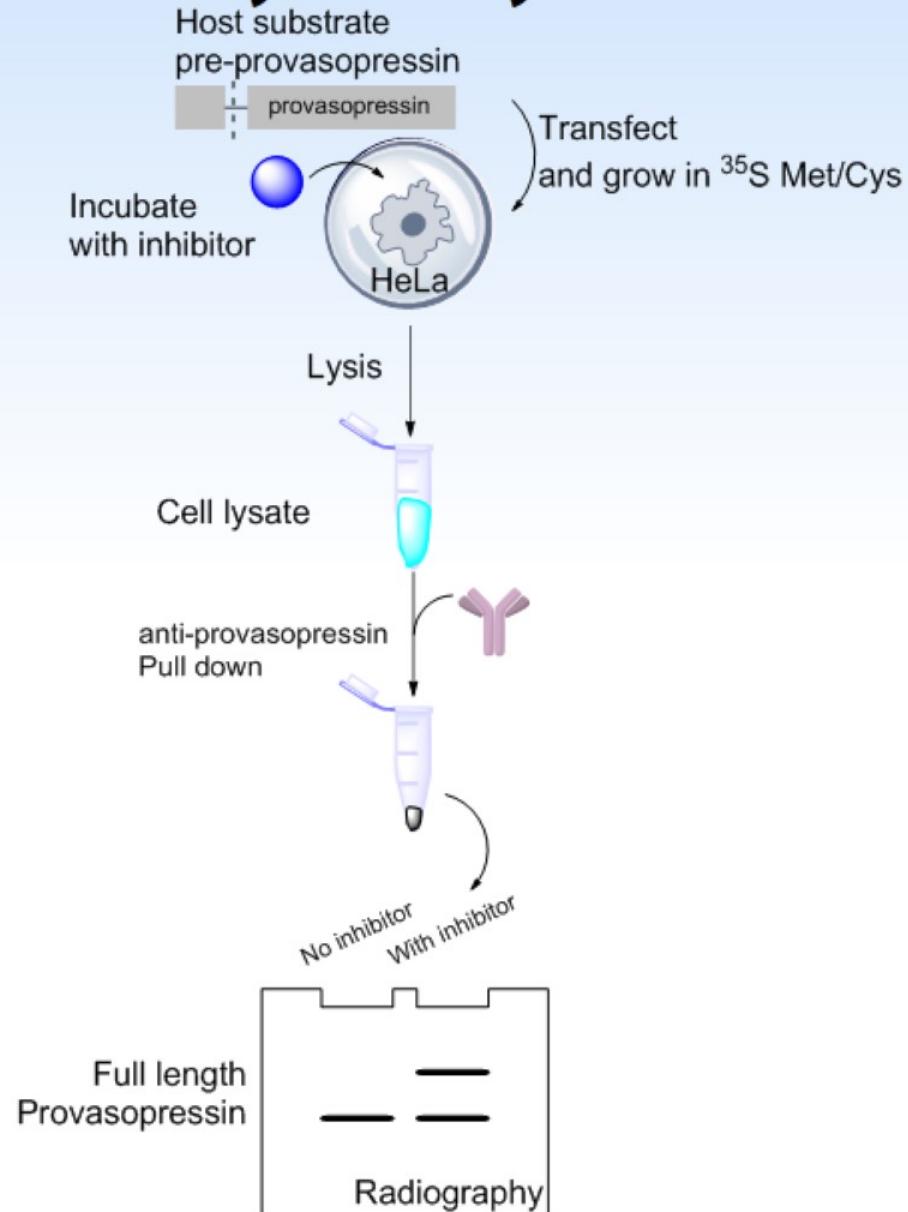
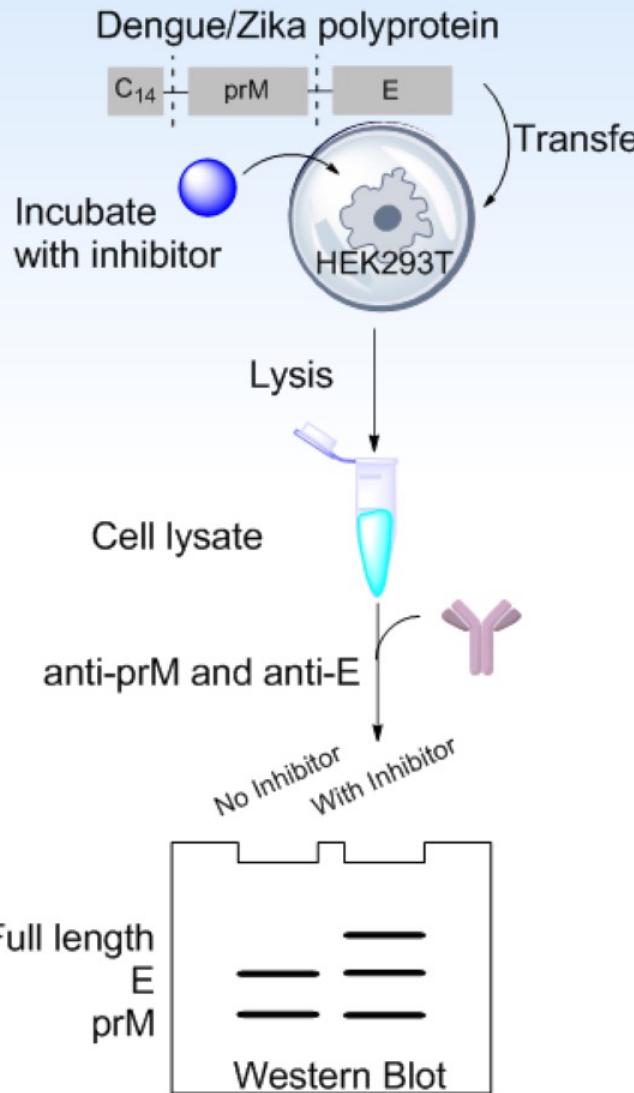
Inhibitor compound



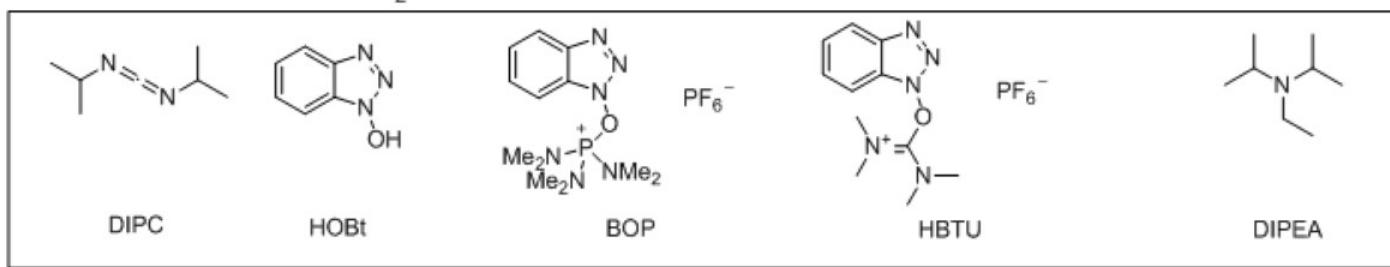
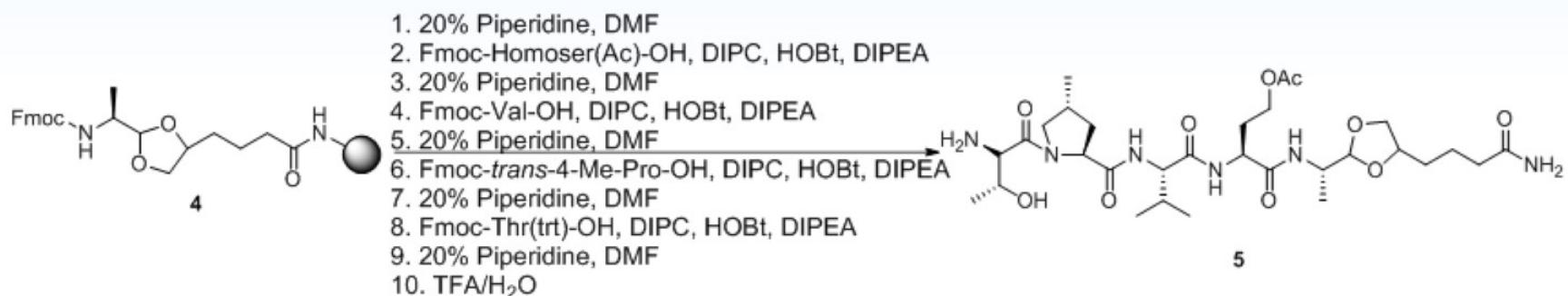
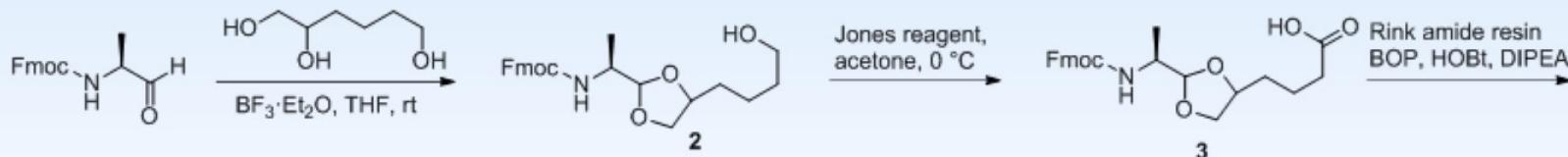
Enzyme - + +
Inhibitor - - +



Cellular activity assays



First CavA Total synthesis



Konno, H., et al., *Tetrahedron* 2015, 71(21), 3433-3438.

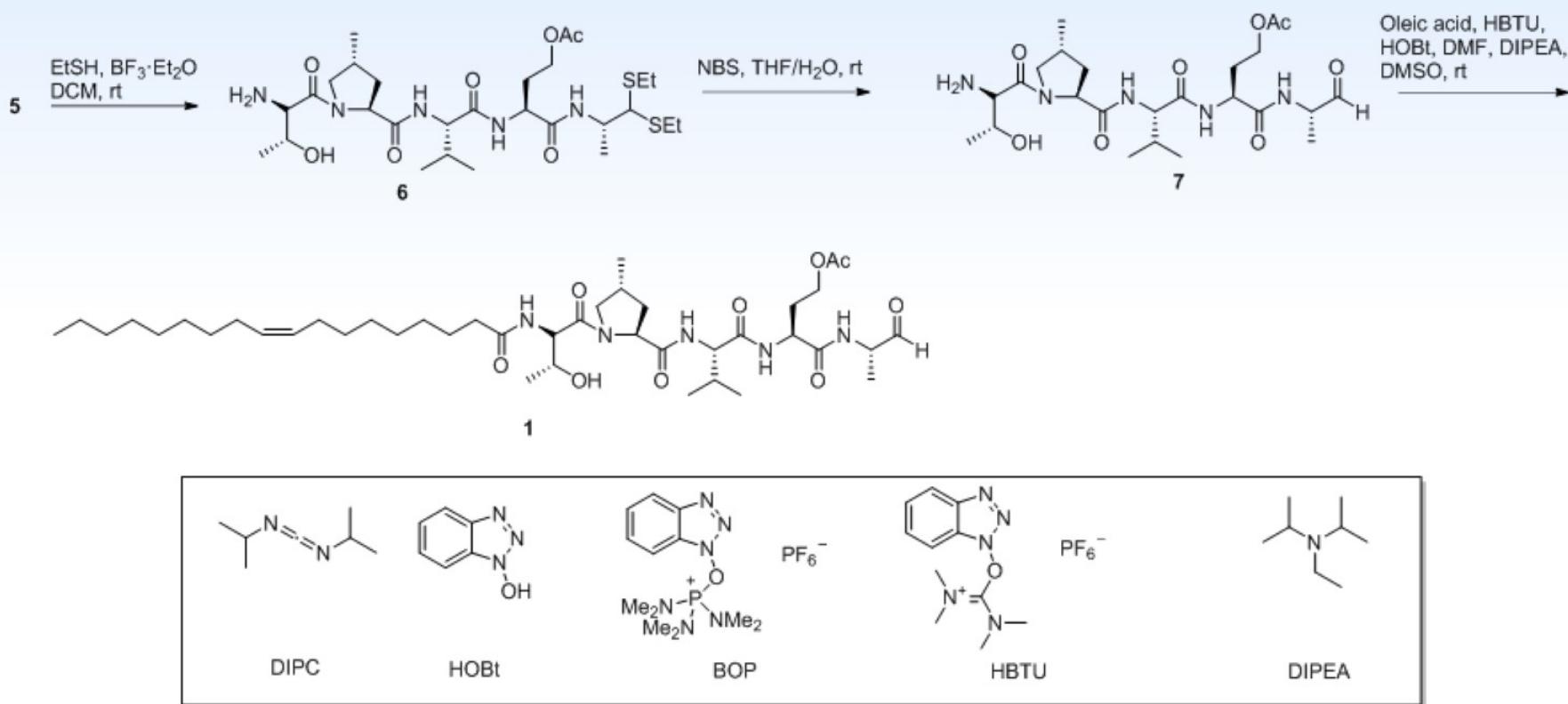
Kinoshita, M., et al., *Bulletin of the Chemical Society of Japan* 1988, 61(6), 2147-2156.

Lallana, E., et al., *Organic Letters* 2006, 8(20), 4449-4452.

Kottani, R., et al., *Proceedings of the National Academy of Sciences* 2006, 103(38), 13917-13921.

Flinn, N. S., et al., *Bioconjugate Chemistry* 2004, 15(5), 1010-1020.

First CavA Total synthesis (2)



Konno, H., et al., *Tetrahedron* 2015, 71(21), 3433-3438.

Kinoshita, M., et al., *Bulletin of the Chemical Society of Japan* 1988, 61(6), 2147-2156.

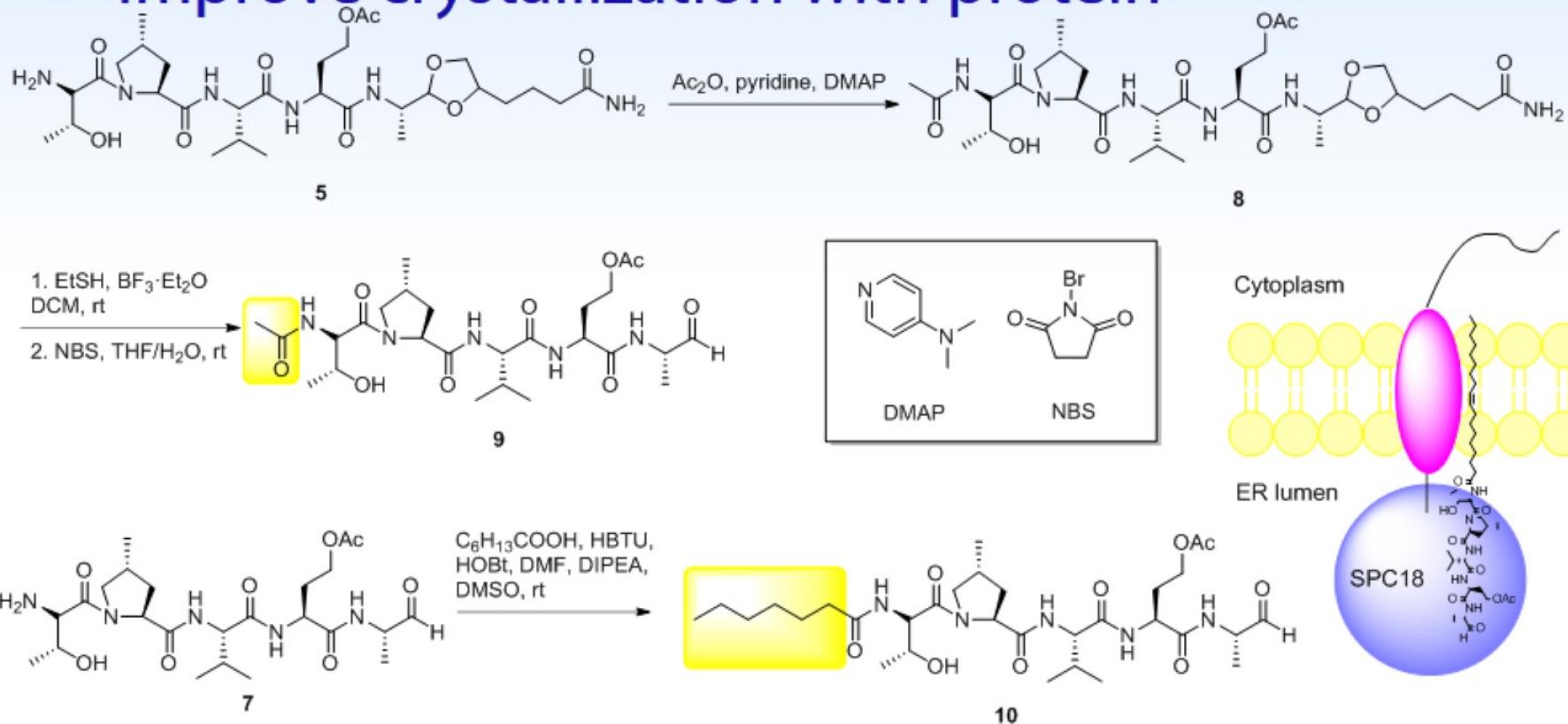
Lallana, E., et al., *Organic Letters* 2006, 8(20), 4449-4452.

Kottani, R., et al., *Proceedings of the National Academy of Sciences* 2006, 103(38), 13917-13921.

Flinn, N. S., et al., *Bioconjugate Chemistry* 2004, 15(5), 1010-1020.

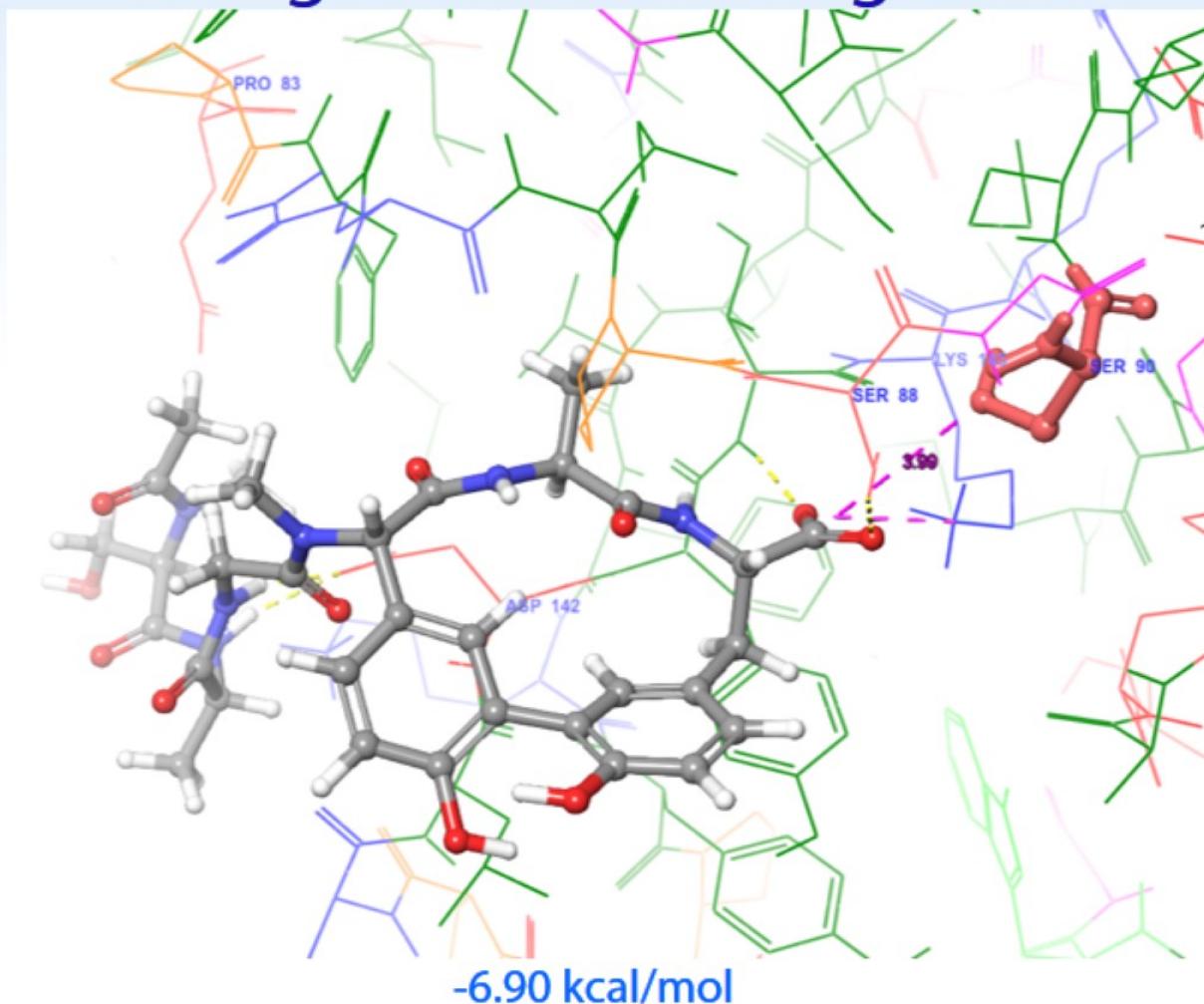
Fatty chain length reduction

- Decrease molecular weight
- Improve crystallization with protein

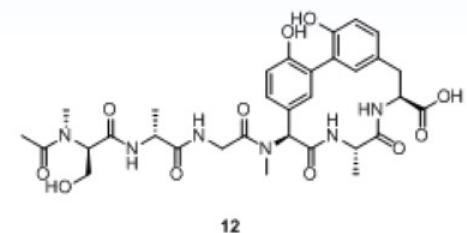


Docking simulation

- Using *E. coli* homolog SPase I

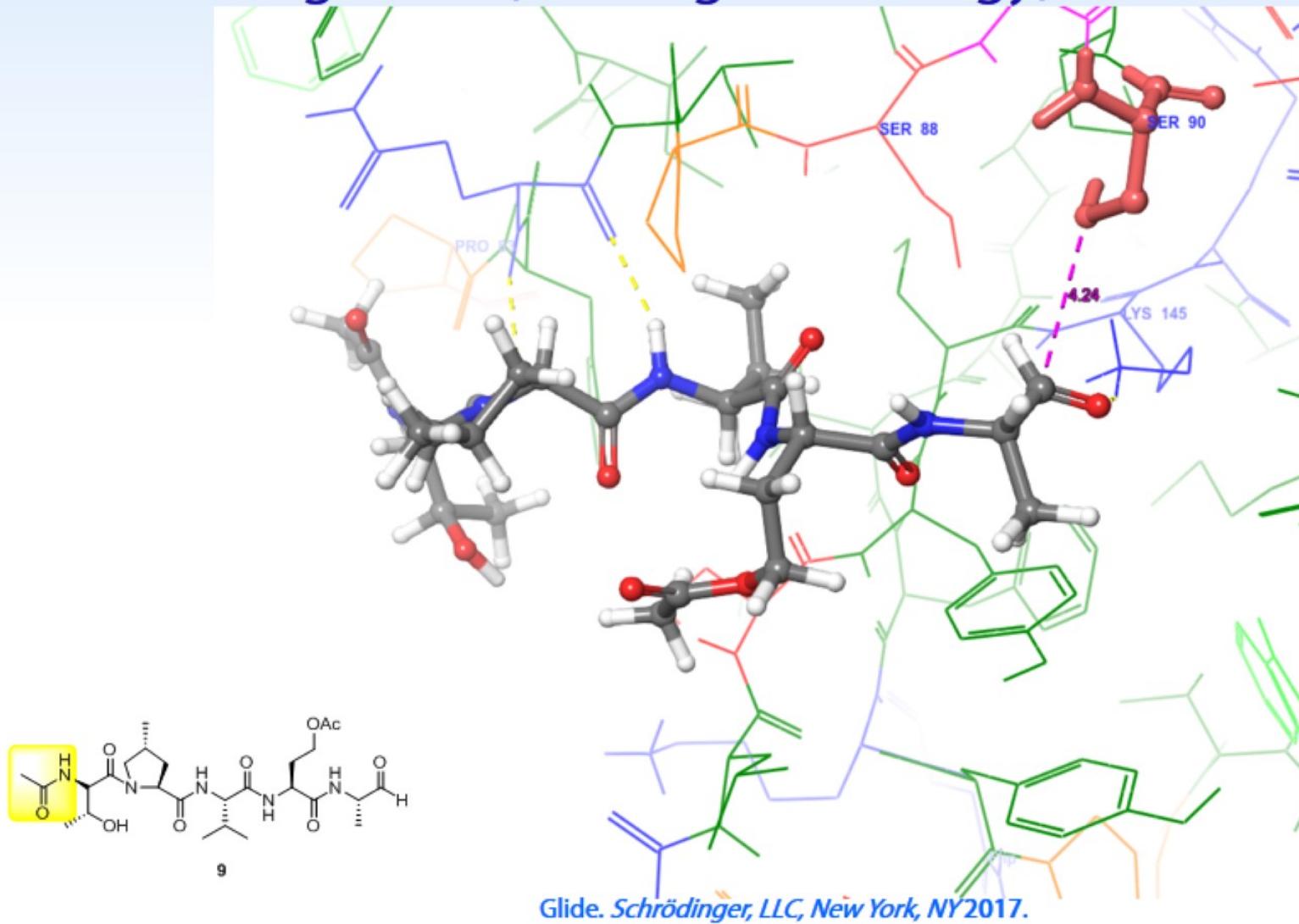


Arylomycin A2 (11)



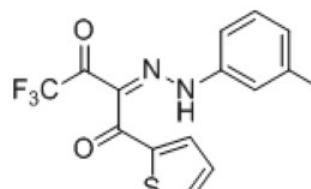
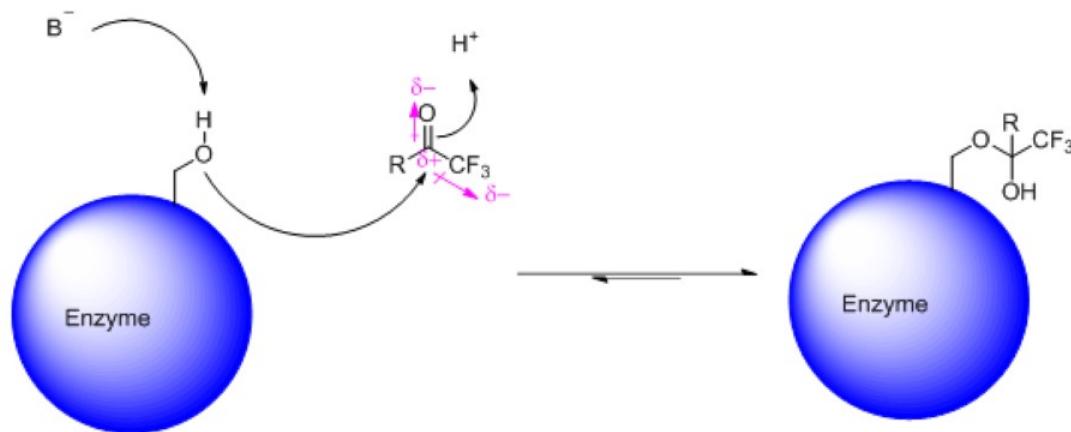
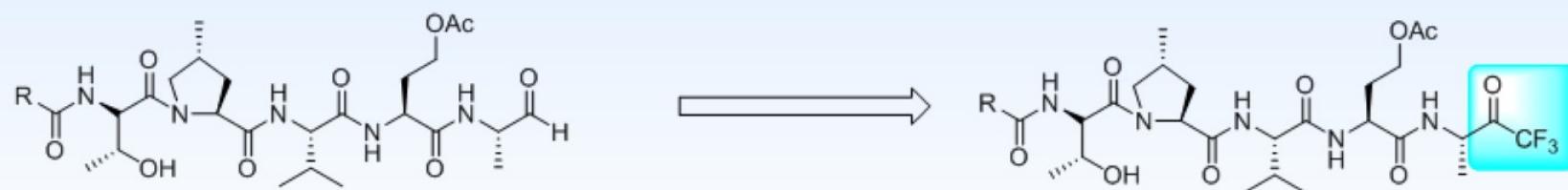
Docking simulation of 9

- Docking score (binding free energy) – -5.70 kcal/mol

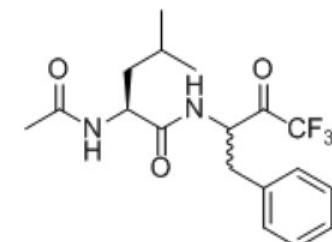


Aldehyde to trifluoromethyl ketone (TMFK)

- Aldehyde – not stable, metabolically reactive



GR148672X



Chymotrypsin Inhibitor
by Abeles et al.

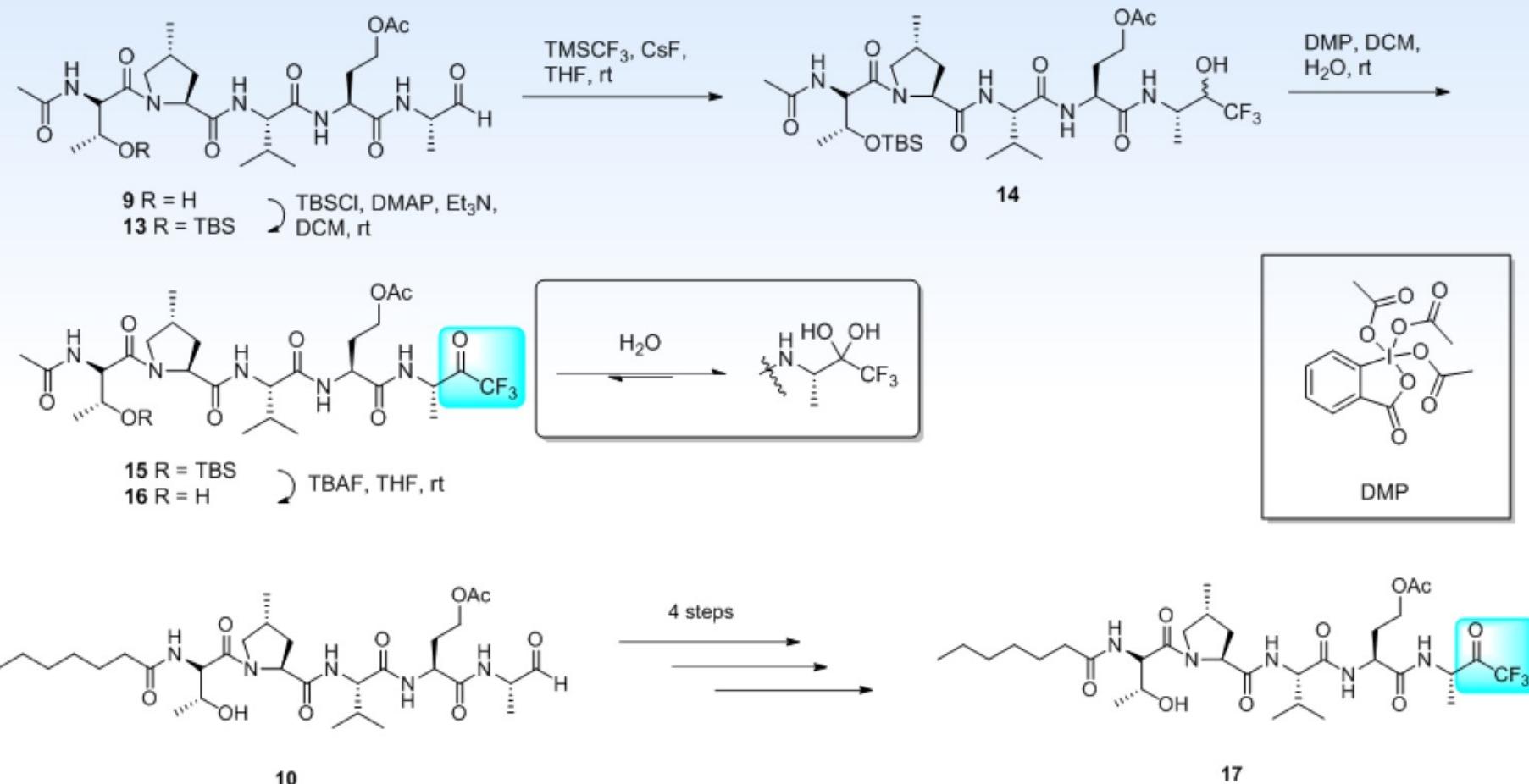
Kelly, C. B., et al., *Chemical Communications* 2013, 49(95), 11133-11148.

Liang, T. C., et al., *Biochemistry* 1987, 26(24), 7603-7608.

Imperiali, B., et al., *Biochemistry* 1986, 25(13), 3760-3767.

Bachovchin, D. A., et al., *Nat Rev Drug Discov* 2012, 11(1), 52-68.

Synthesis of 16 and 17



Prakash, G. K. S., et al., *Chemical Reviews* 1997, 97(3), 757-786.

Leon, R., et al., *Chemical Science* 2011, 2(8), 1487-1490.

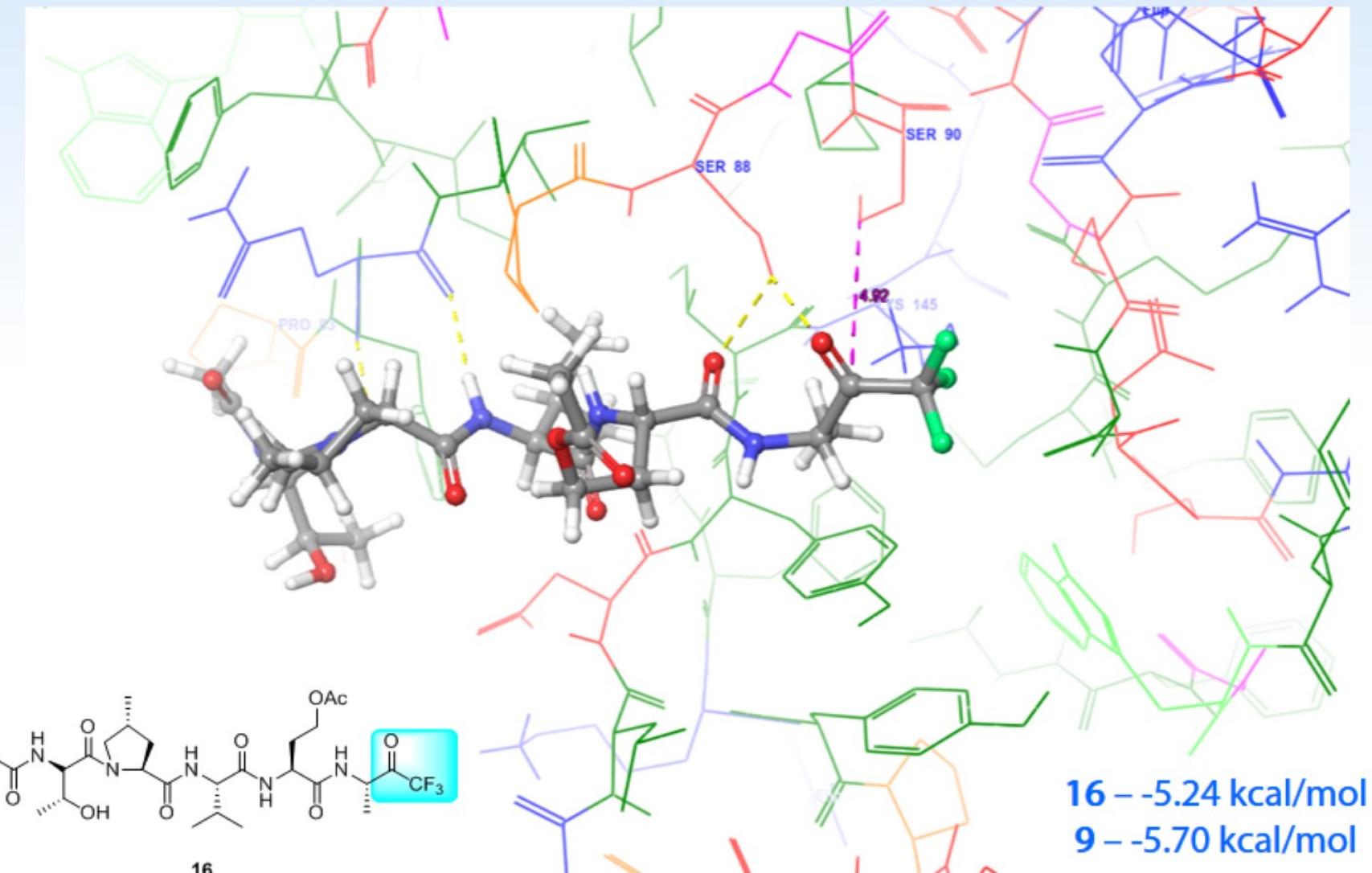
Walter, M. W., et al., *Bioorganic & Medicinal Chemistry Letters* 1996, 6(20), 2455-2458.

Inglis, S. R., et al., *Journal of Medicinal Chemistry* 2009, 52(19), 6097-6106.

Imperiali, B., et al., *Biochemistry* 1986, 25(13), 3760-3767.

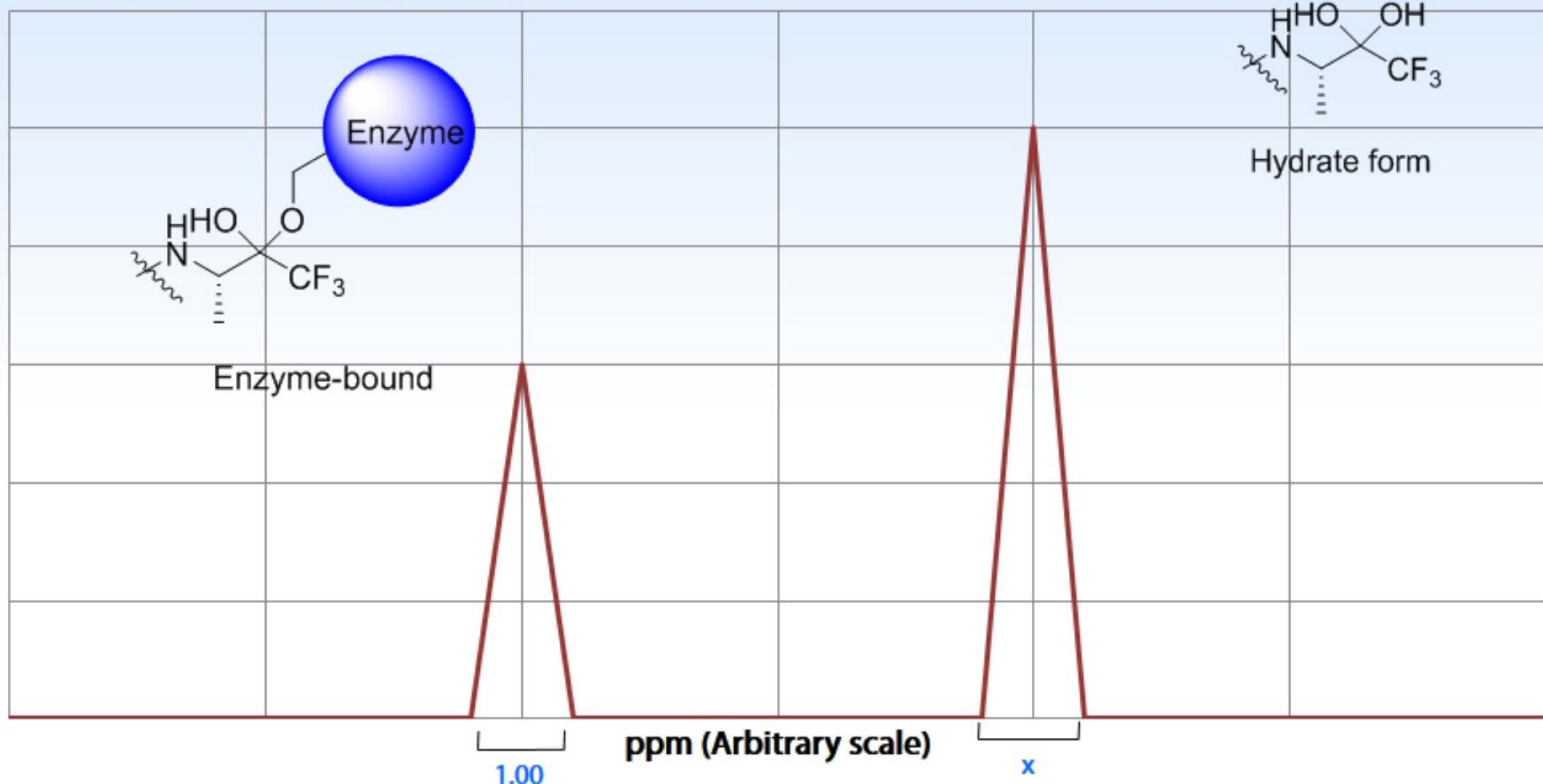
Rodeschini, V., et al., *The Journal of Organic Chemistry* 2005, 70(6), 2409-2412.

Docking simulation of 16



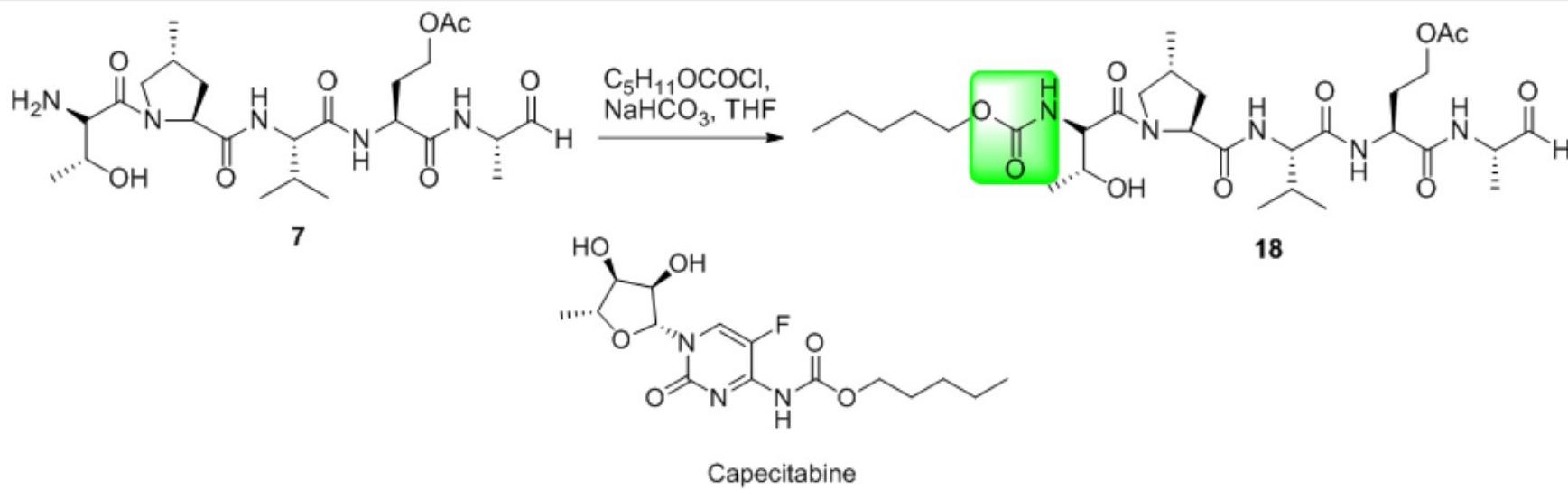
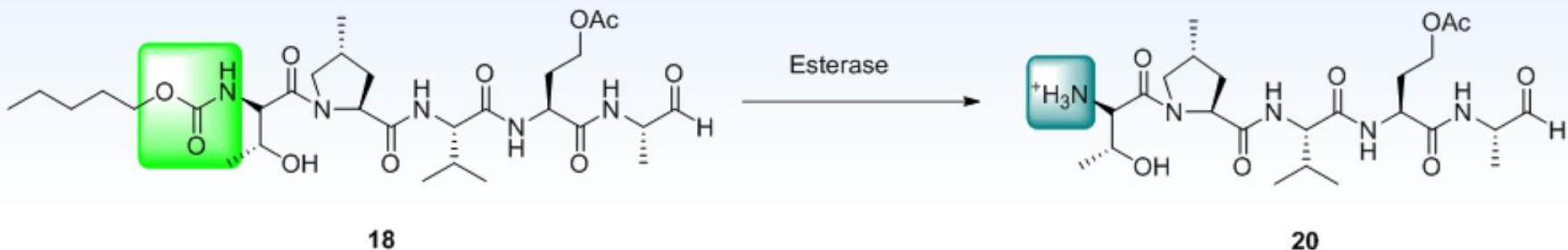
^{19}F NMR assay

Sample ^{19}F NMR spectrum



Carbamate moiety

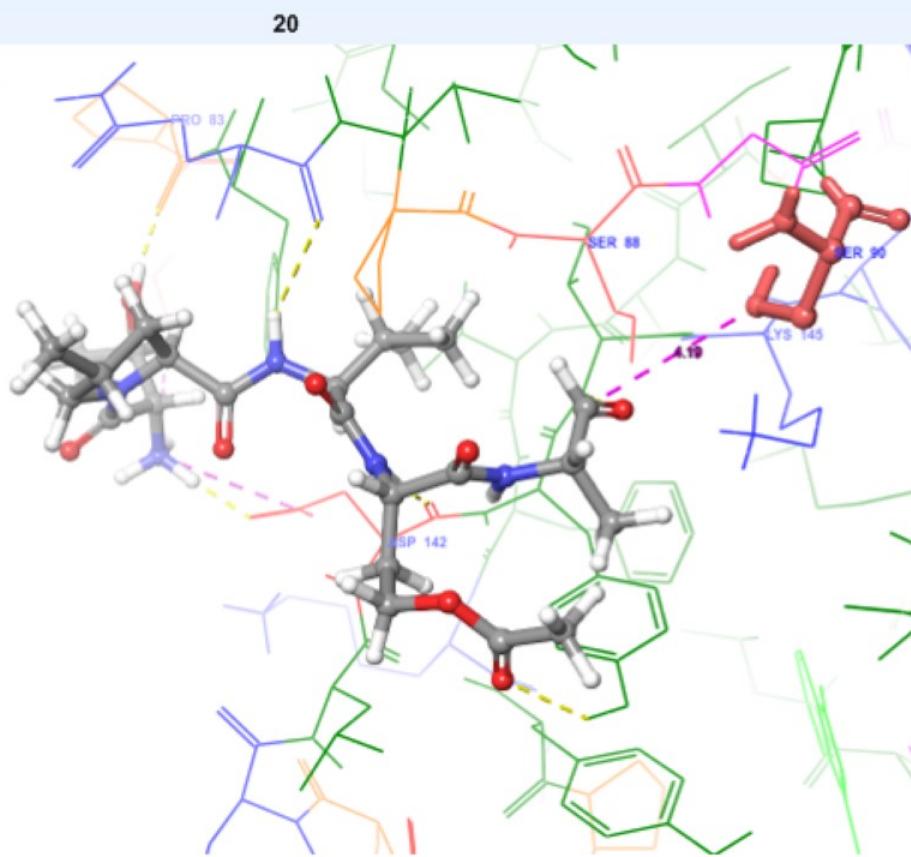
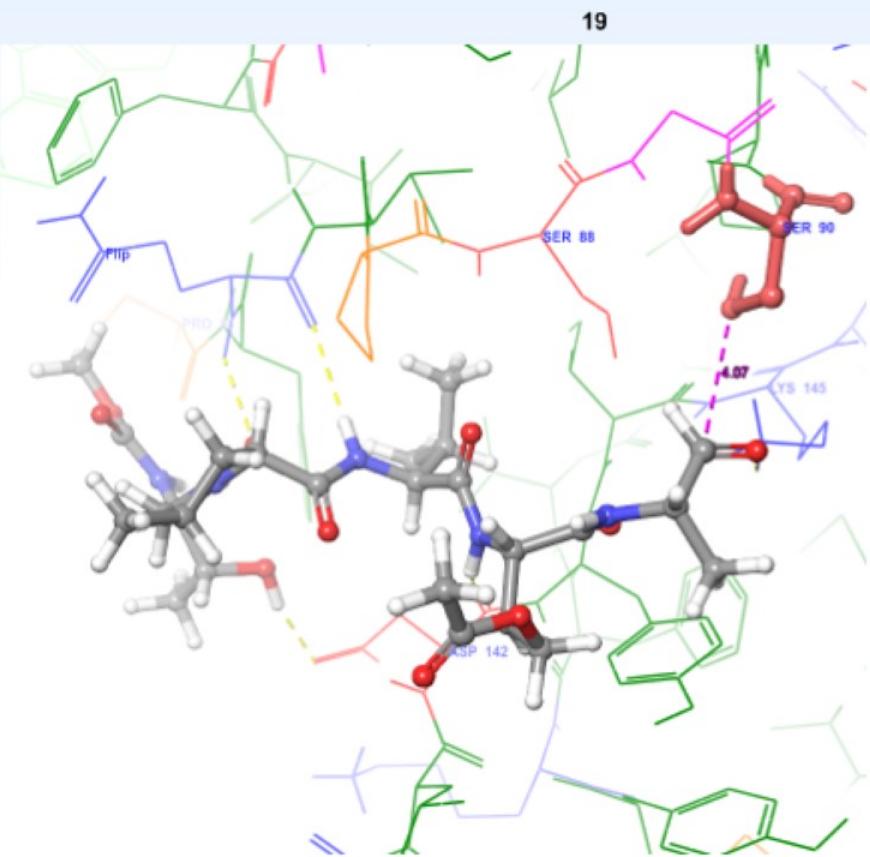
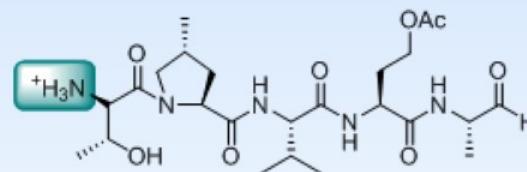
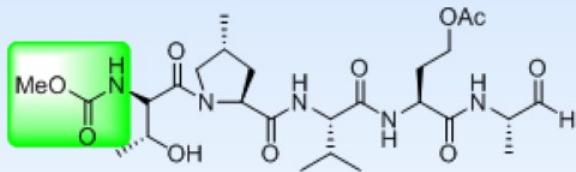
- Prodrug – Modified drug molecule undergoing transformation *in vivo* to release active drug
 - Increase membrane permeability/bioavailability



Rautio, J. et al., *Nat Rev Drug Discov* 2008, 7(3), 255-270.

Ghosh, A. K. et al., *Journal of Medicinal Chemistry* 2015, 58(7), 2895-2940.

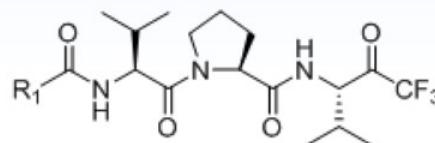
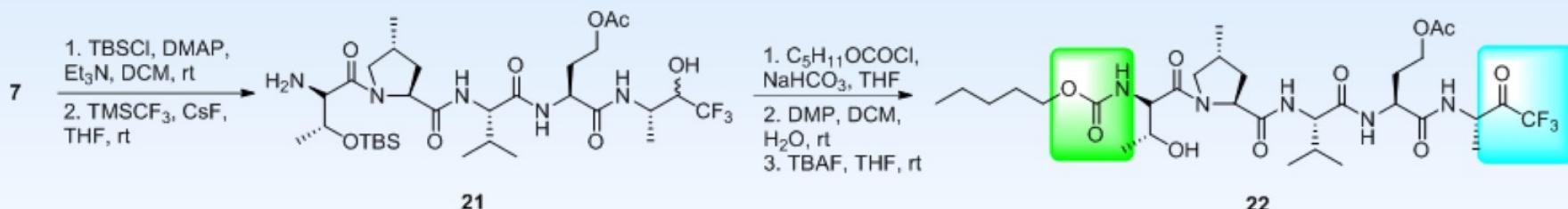
Carbamate docking simulations



-5.59 kcal/mol

-7.42 kcal/mol

Carbamate + TMFK moieties



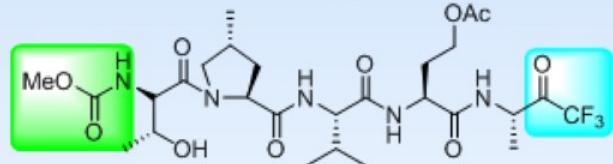
Elastase inhibitors, AstraZeneca®

compd	R ₁	stereochemistry at P1 position	molecular formula ^a	<i>K</i> _i (nM) ^b	acute hemorrhagic assay		
					% inhibition, ^c 10 mg/kg oral dose	intravenous ED ₅₀ (mg/kg)	oral ED ₅₀ (mg/kg)
1k	4-MeOC ₆ H ₅	<i>S</i>	C ₂₄ H ₃₂ N ₃ O ₅ F ₃	1.3 ± 0.3	84	0.59	4.9
1l	CH ₃ O	<i>S</i>	C ₁₈ H ₂₈ N ₃ O ₅ F ₃ ·1.0H ₂ O	13 ± 1.7	92	0.51	2.0
1m	isopropyl-O	<i>S</i>	C ₂₀ H ₃₂ N ₃ O ₅ F ₃ ·1.5H ₂ O	5.8 ± 1.3	80		3.0

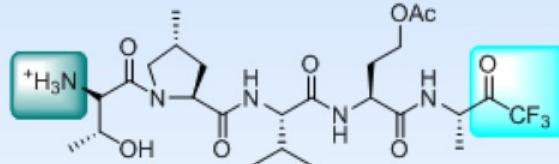
^a All compounds were analyzed for C, H, N; the results agreed to within $\pm 0.4\%$ of the theoretical values. ^b Method reported in ref 25.

^c Percent inhibition of elastase-induced lung damage when the compound was dosed orally at 10 mg/kg, 30 min prior to the instillation of a 50 μ g challenge of elastase.

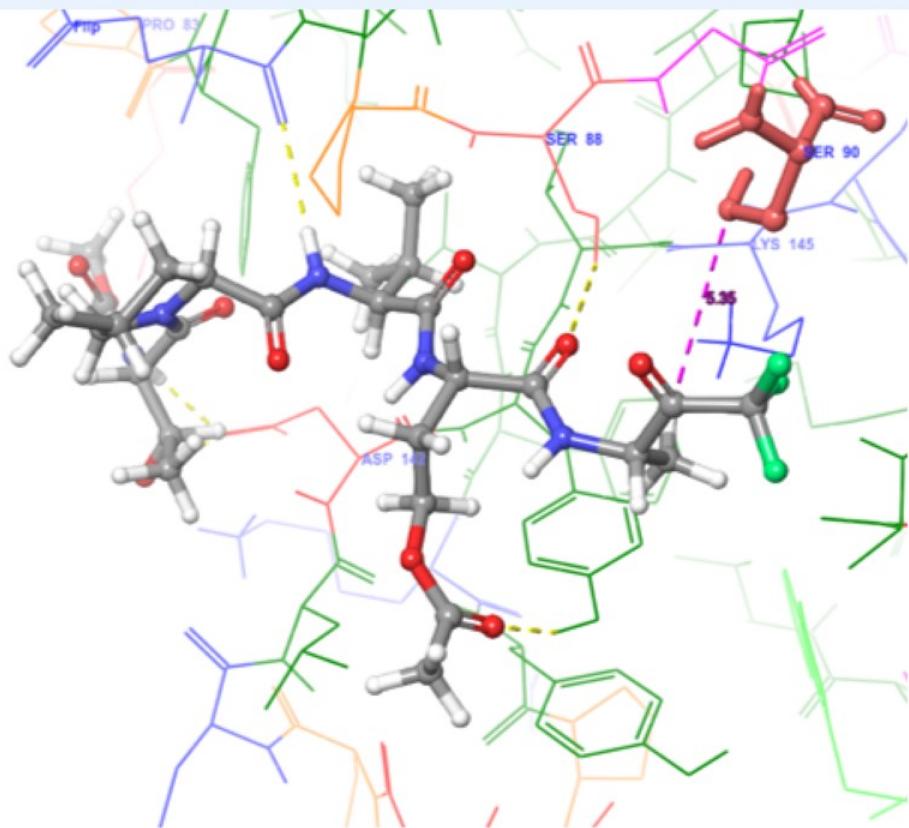
Docking simulations of 23, 24



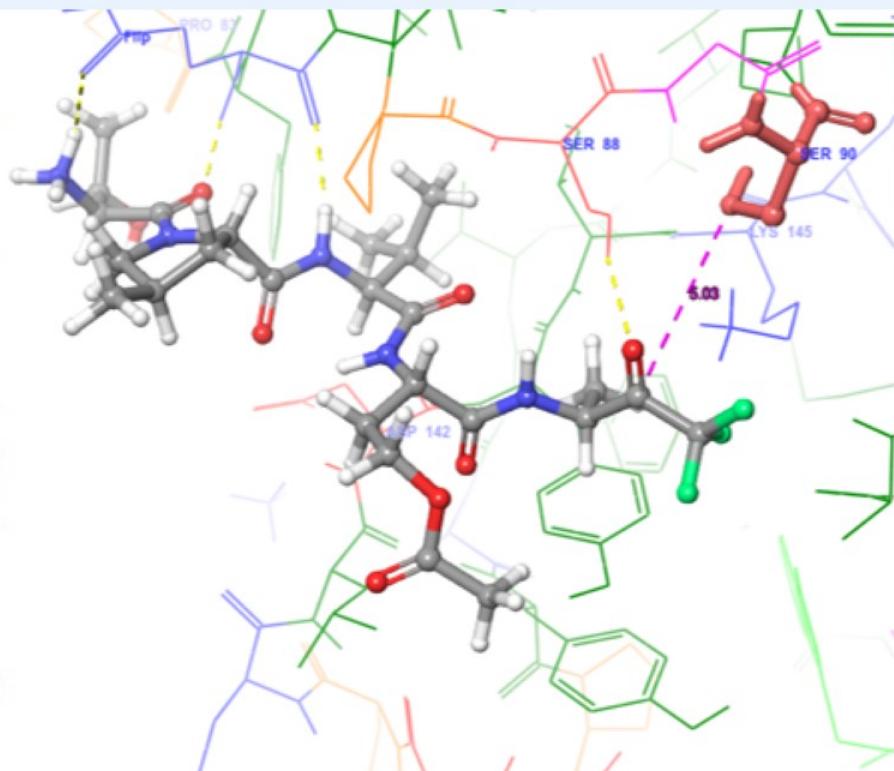
23



24



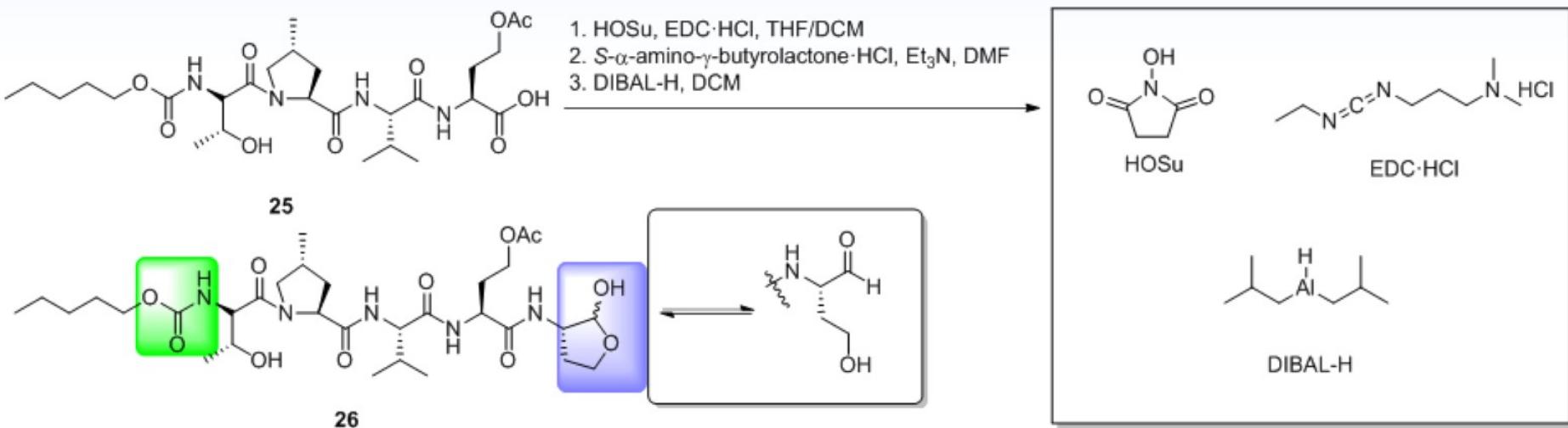
-6.76 kcal/mol



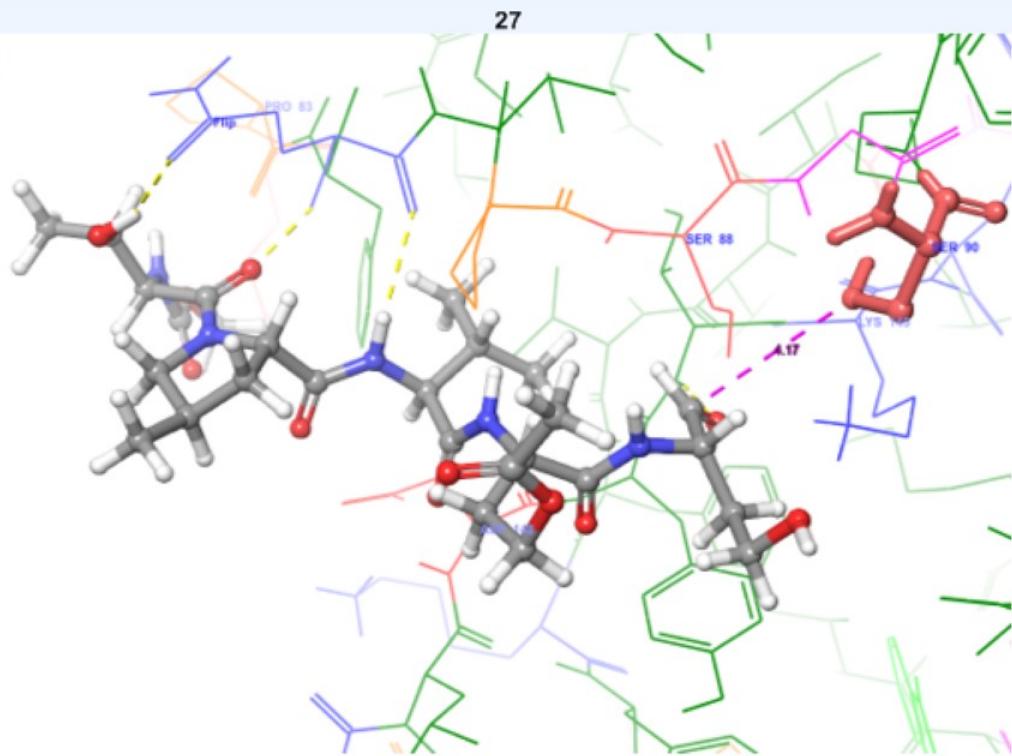
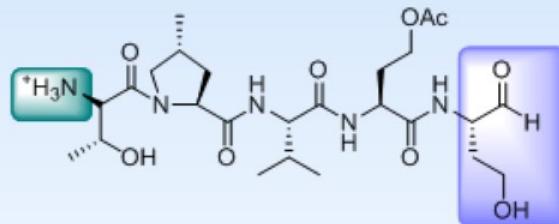
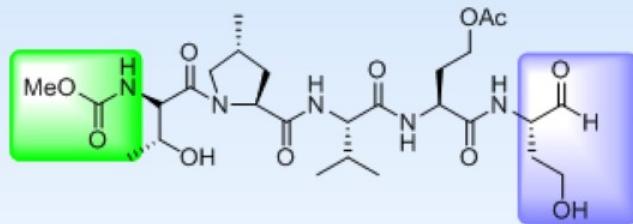
-5.76 kcal/mol

Cyclic hemiacetal moiety

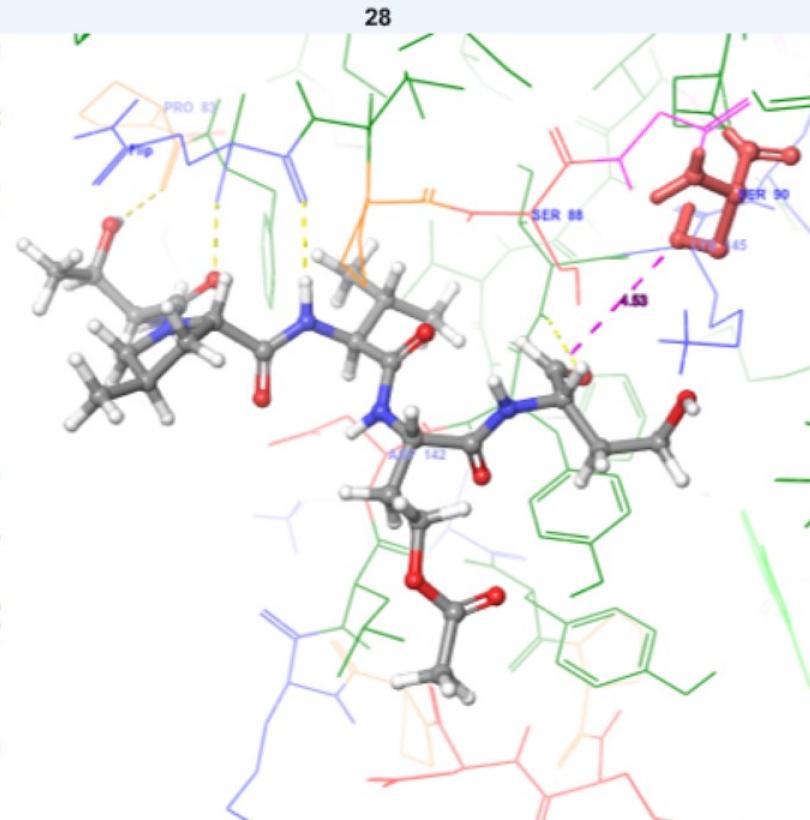
- Alternative to TMFK
- Binding relies on equilibrium



Hemiacetal docking simulations

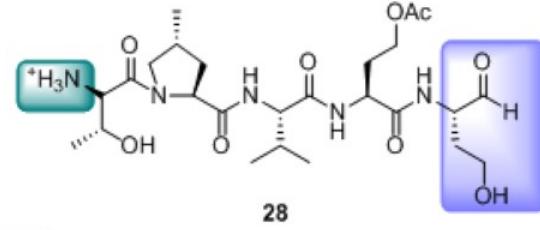
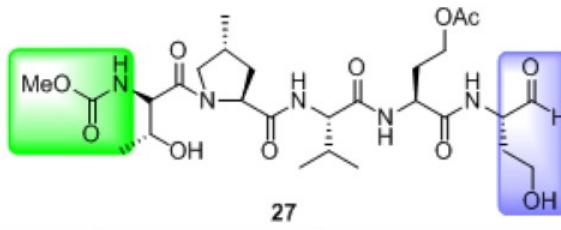
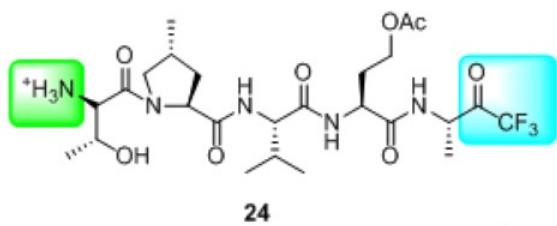
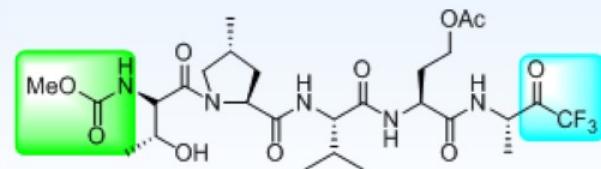
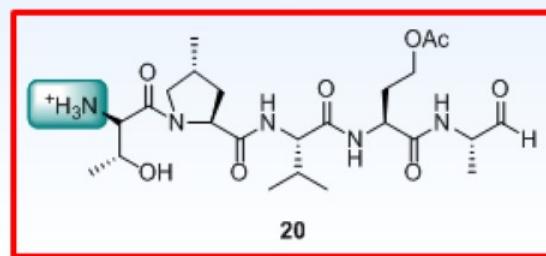
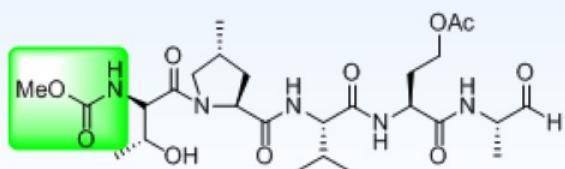
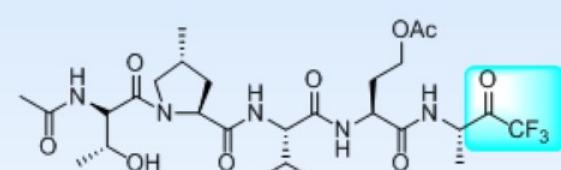
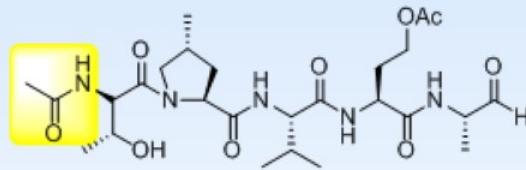
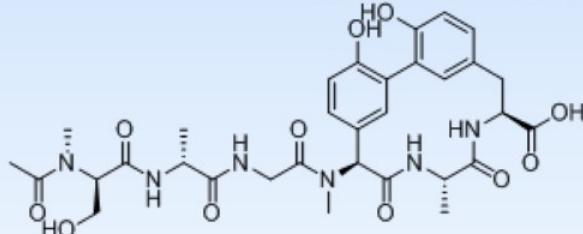


-5.14 kcal/mol



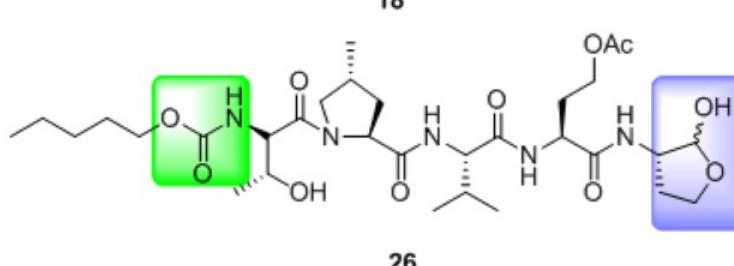
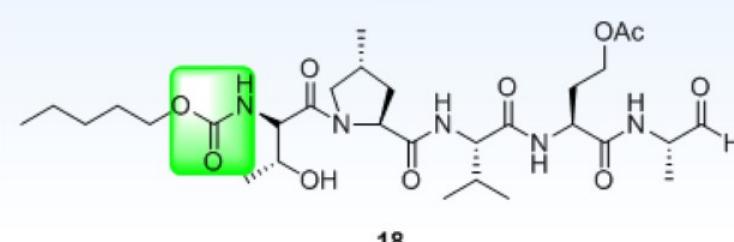
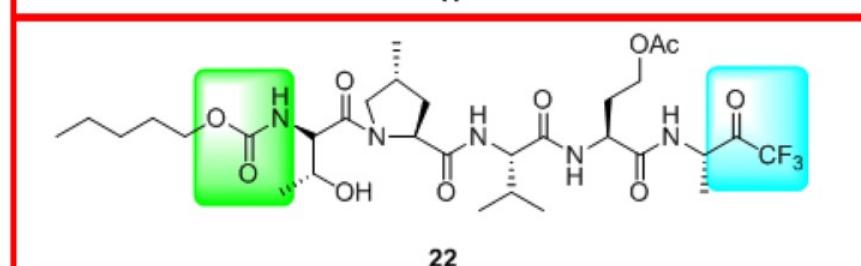
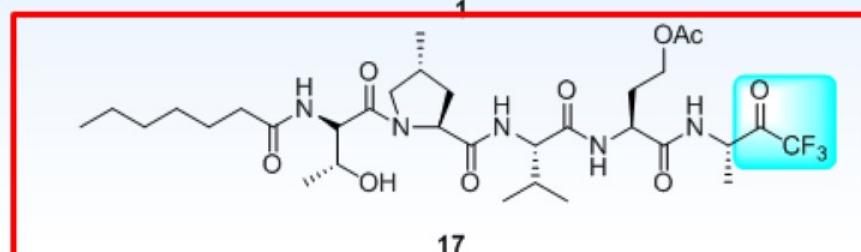
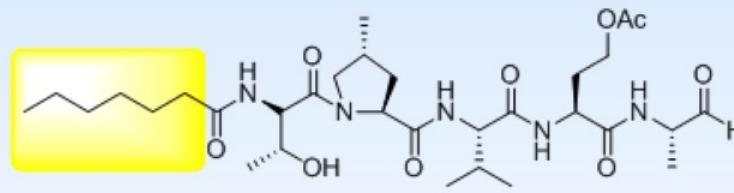
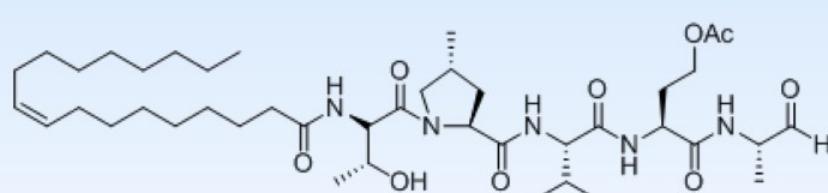
-4.84 kcal/mol

Docking results summary



Compound	Score (kcal/mol)	Ser90 to C=O Distance (Å)
12 (control)	-6.90	3.99
9	-5.70	4.24
16	-5.24	4.92
19	-5.59	4.07
20	-7.42	4.19
23	-6.76	5.35
24	-5.76	5.03
27	-5.14	4.17
28	-4.84	4.53

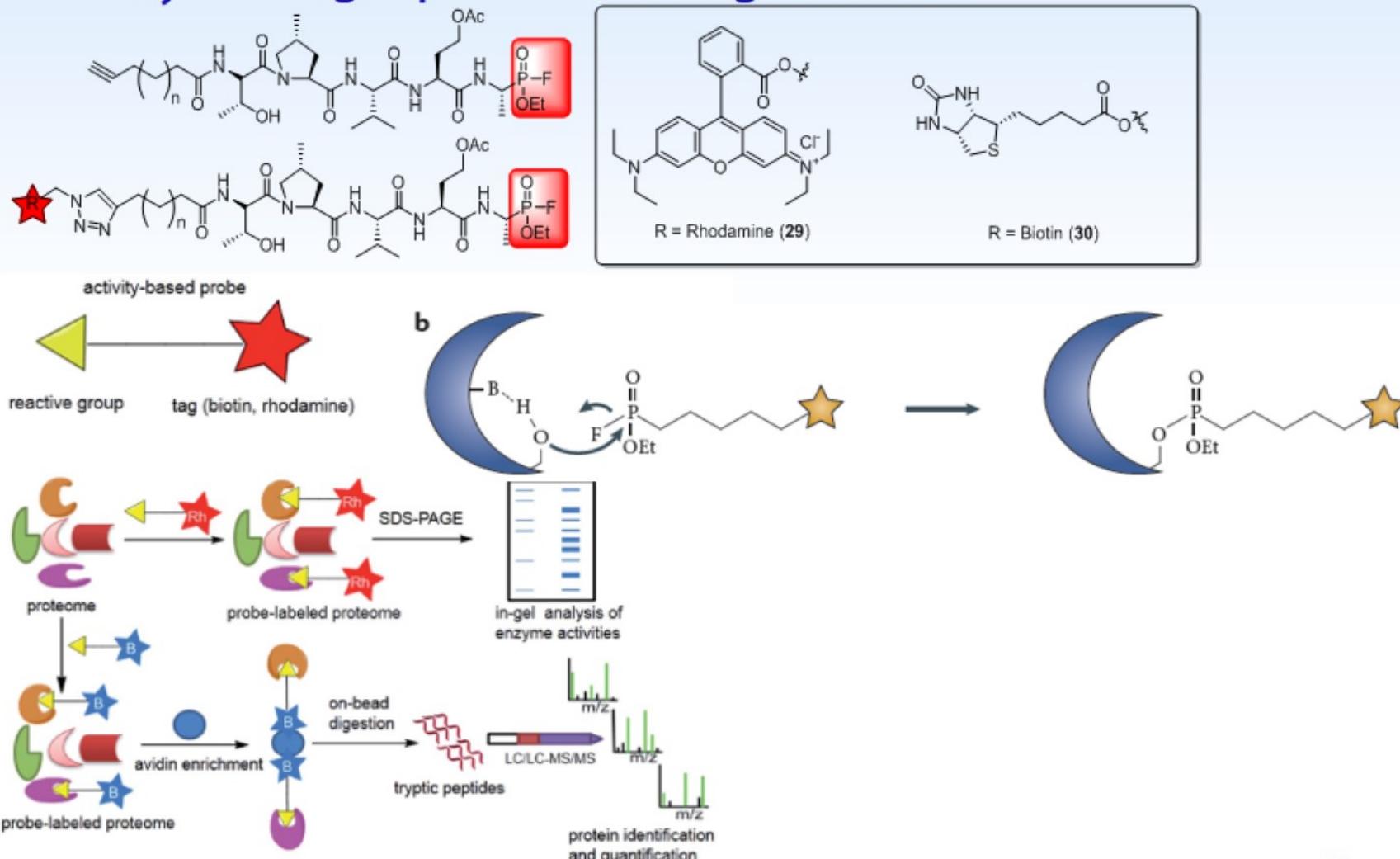
Predicted ADMET properties



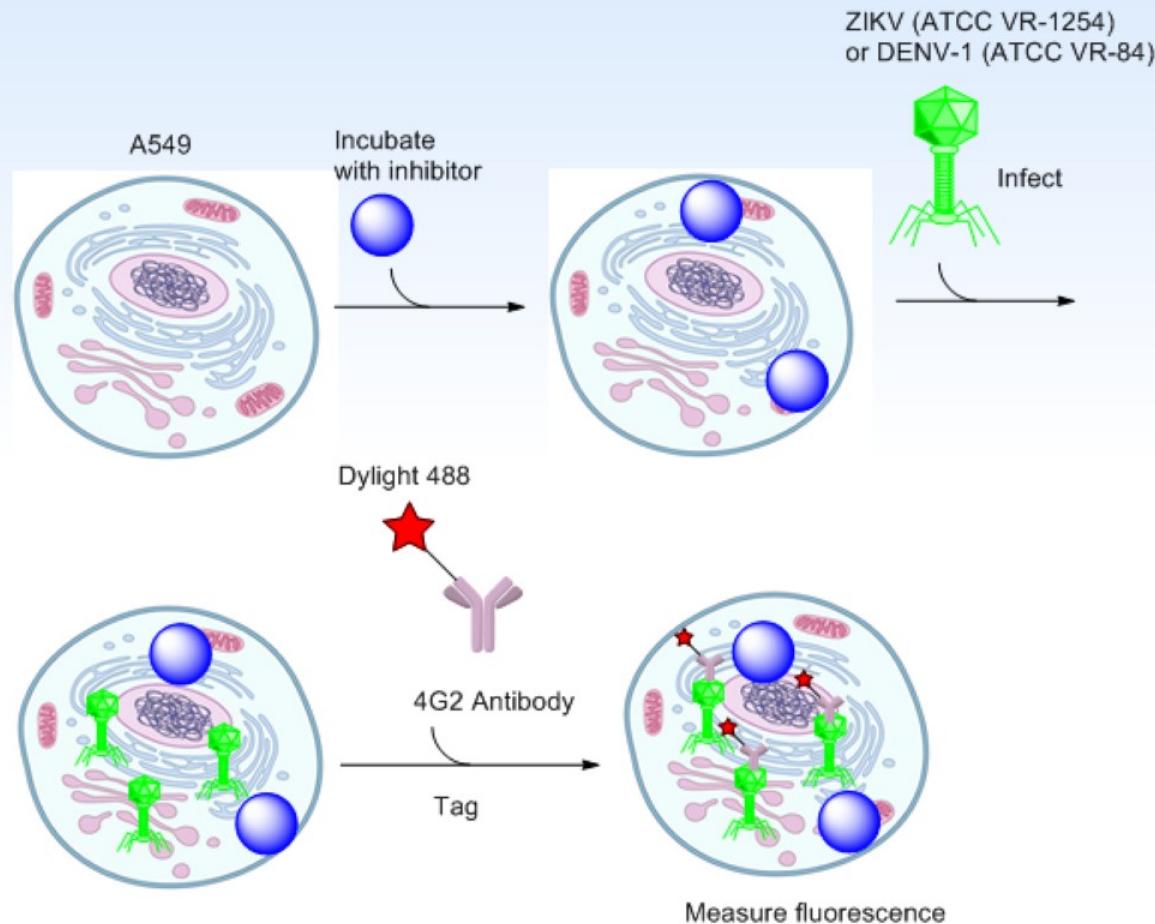
molecule	mol_MW	#rotor	SASA	volume	QPlogPo/w	QPPCaco	PercentHumanOralAbsorption	SAfluorine	RuleOfFive	RuleOfThree
1	792.067	30	1406.8	2681.822	5.04	11.249		36.395	0	3
10	639.788	20	1052.938	2036.24	1.007	11.604		25.982	0	2
17	707.786	20	1140.956	2158.754	2.388	37.218		43.126	107.501	2
18	641.76	19	1035.453	2008.724	1.522	20.434		33.393	0	2
22	709.759	19	1119.671	2130.821	2.772	36.851		45.297	101.433	2
26	671.787	19	1072.578	2064.217	0.844	25.283		18.117	0	3

Activity-based probes

- Identify off-target protein binding activities

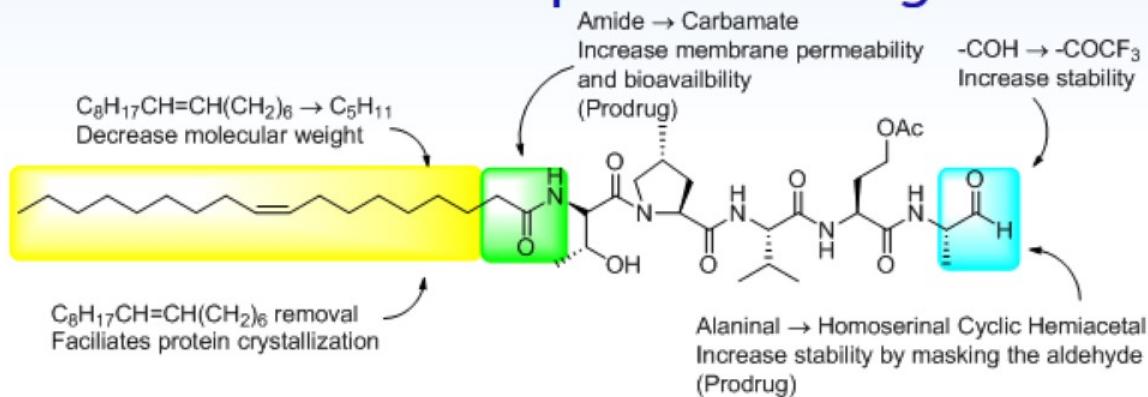


Live virus assays

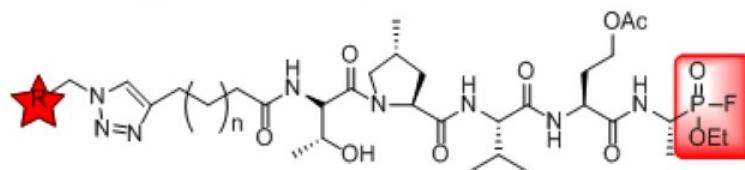


Summary

- Zika and dengue fever affects worldwide population with no specific treatment available
- First Cavinafungin A total synthesis proposed
- CavA derivatives are potential drug candidates



- Activity-based probes identifies off-target bindings



END OF PRESENTATION

More about Zika virus

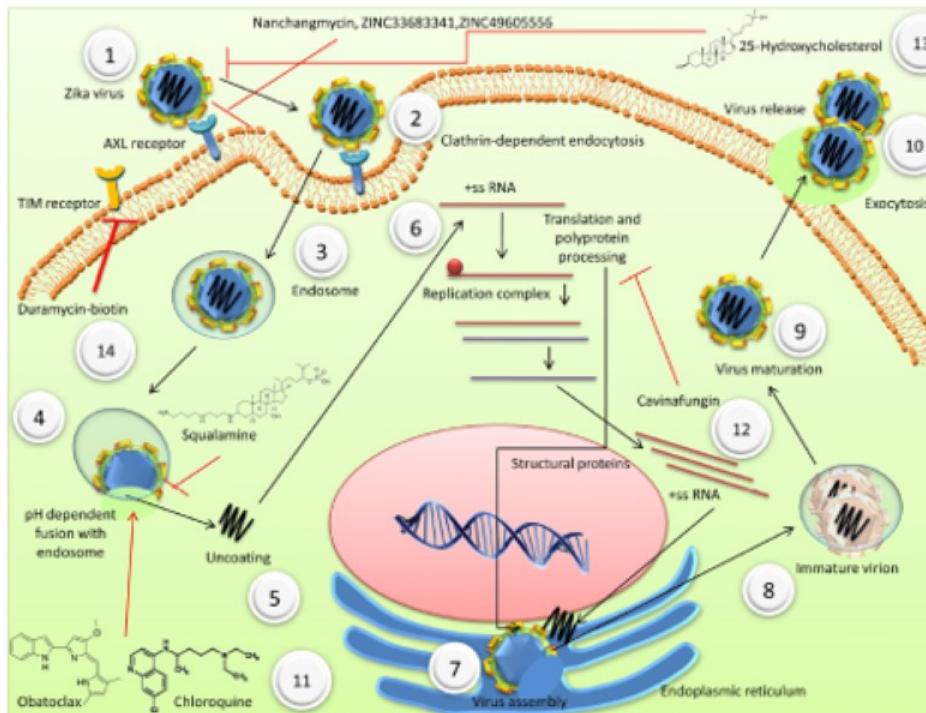


FIGURE 1 | Mode of entry of Zika virus (ZIKV) and various drugs inhibiting viral entry and replication (1) ZIKV binds to cell receptors including AXL, DC-SIGN, Tyro3, TIM, and TAM. (2) Clathrin-dependent endocytosis. (3) Endosome mediated transport of ZIKV. (4) Fusion of virus membrane with host endosomal membrane, which depends on the pH. (5) Uncoating (6) The positive-sense genomic ssRNA is translated into a polyprotein, which is cleaved into all structural and non-structural proteins. Replication occurs at the surface of endoplasmic reticulum in cytoplasmic viral factories. A dsRNA genome is synthesized from the genomic ssRNA(+) (7) Virus assembly takes place at the endoplasmic reticulum. (8) At the endoplasmic reticulum, virions bud and are transported to the golgi apparatus. (9) In the golgi, prM protein is cleaved and maturation of the virion takes places. (10) Virions are released by exocytosis. (11) Obatoclax and chloroquine inhibit the acidic environment of endolysosomal vesicles. Squalamine, a cationic chemical, disturbs the electrostatic interaction between virus and host membranes during fusion and budding. (12) Cavinafungin, an alanine-containing lipopeptide of fungal origin, inhibits ZIKV polyprotein processing and also the cleavage of signal peptide of host proteins. (13) Nanchangmycin, a polyether obtained from *Streptomyces nanchangensis*; small drug-like molecules, ZINC33683341 and ZINC49605556 block the receptor thus inhibiting the ZIKV entry. (14) TIM1 mediated entry is inhibited by Duramycin-biotin.

Proteins of Zika/dengue

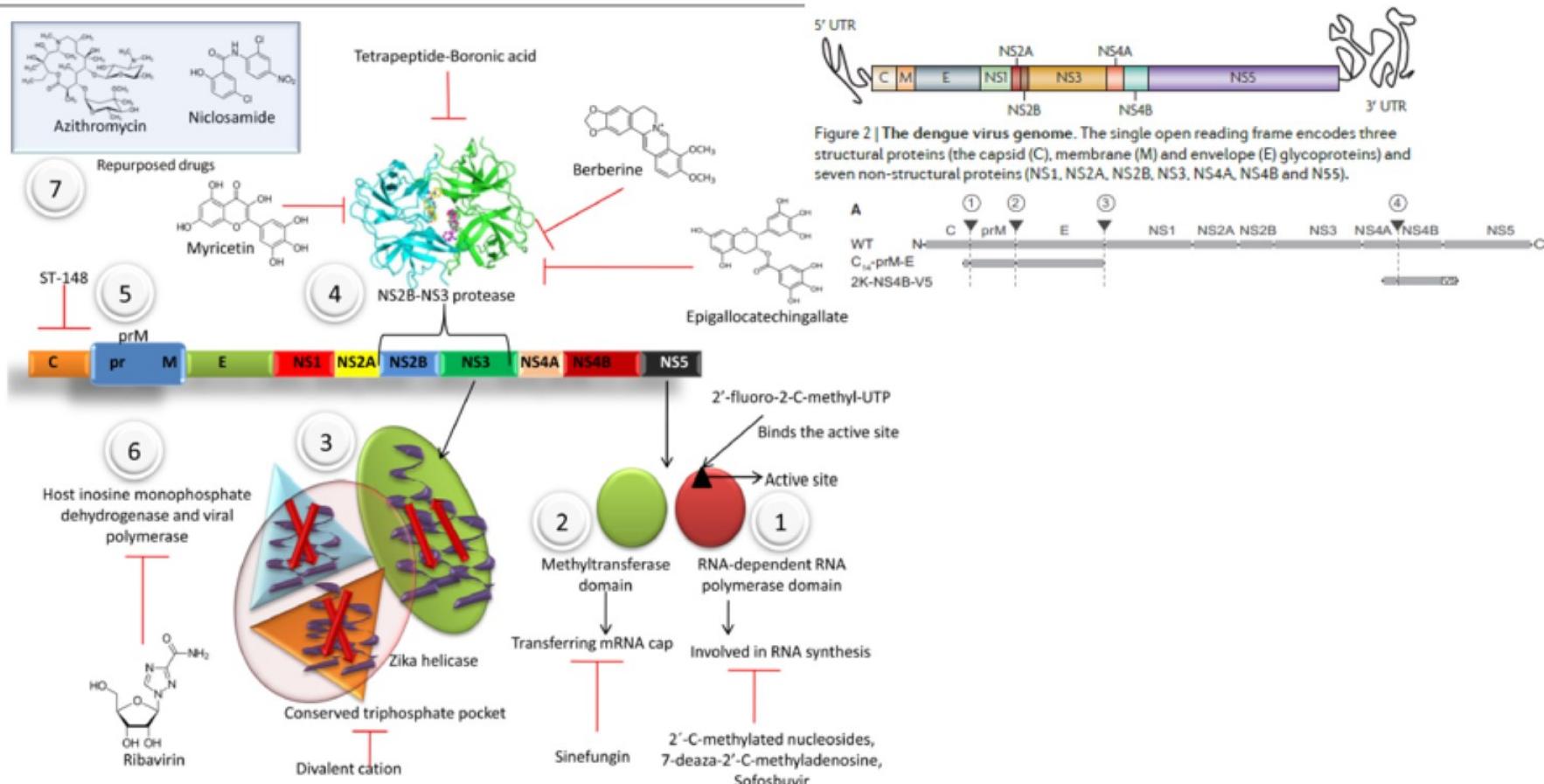
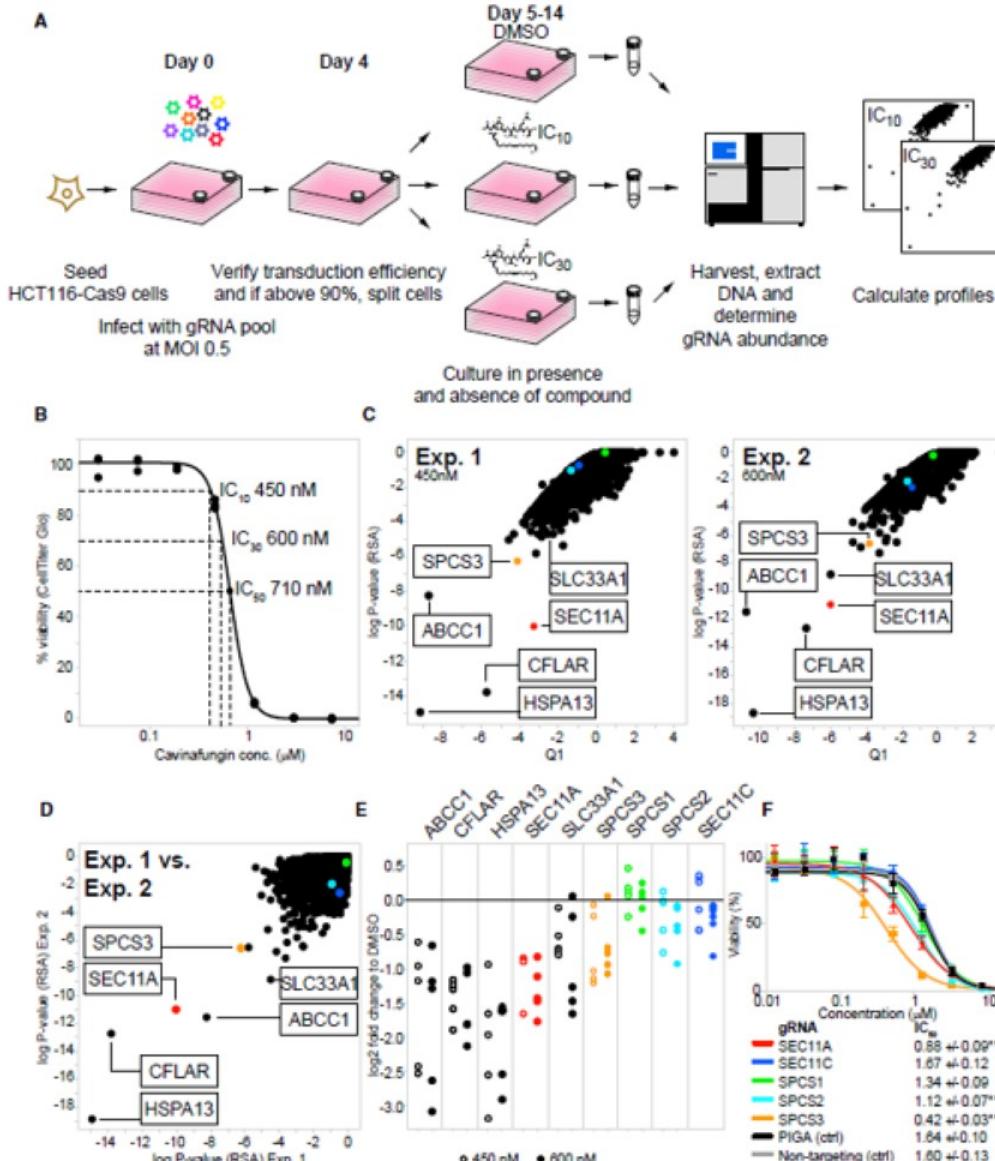


FIGURE 2 | Various drugs involved in inhibition of virus replication at different stages. (1) Flaviviral NS5 has two major catalytic domains: RNA-dependent RNA polymerase (RdRp) and methyltransferase domain. Nucleoside analogs like 2'-C-methylated nucleosides, 7-deaza-2'-C-methyladenosine, Sofosbuvir may incorporate during the polymerase activity of RdRp in the viral nascent RNA chain and cause premature termination of RNA synthesis. The 2'-fluoro-2-C-methyl-UTP binds to the active site on NS5. (2) Methyltransferase domain is responsible for transferring mRNA cap. Sinefungin, an adenosine derivative, isolated from *Streptomyces griseoileus*, inhibit S-adenosyl-1-methionine (SAM), the natural substrate for methyltransferases and inhibit the methyltransferase activity. (3) Helicase crystal structure reveals a conserved triphosphate pocket and a positively charged tunnel for the accommodation of RNA. The helicase-activation is inhibited in the presence of divalent cation, due to extended conformation adopted by GTPyS in such conditions. (4) Tetrapeptide-Boronic acid is a potent inhibitor of NS2B-NS3 protease. Berberine, Myricetin, Epigallocatechingallate binds with affinity to NS3 protease and also inhibit the ZIKV replication. (5) Small-molecule inhibitor ST-148 inhibits capsid. (6) Ribavirin inhibits host inosine monophosphate dehydrogenase and viral polymerase. (7) Repurposed drugs like Chloroquine, azithromycin, niclosamide are used to treat ZIKV infection.

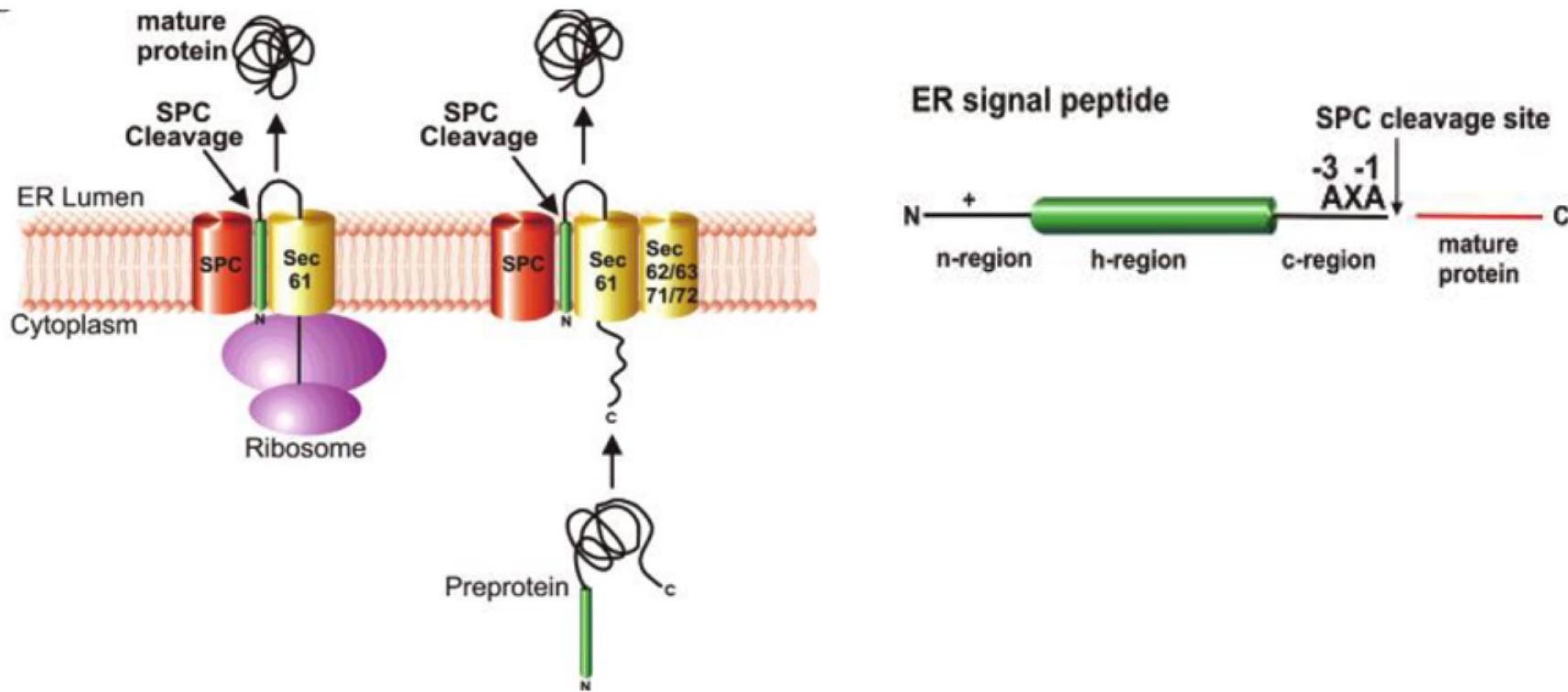
CavA CRISPR profiling



Estoppey, D., et al., *Cell Reports* 2017, 19(3), 451-460.

Lee, A.Y., et al., *Science* 2014, 344(6180), 208-211.

Eukaryotic signal peptidase



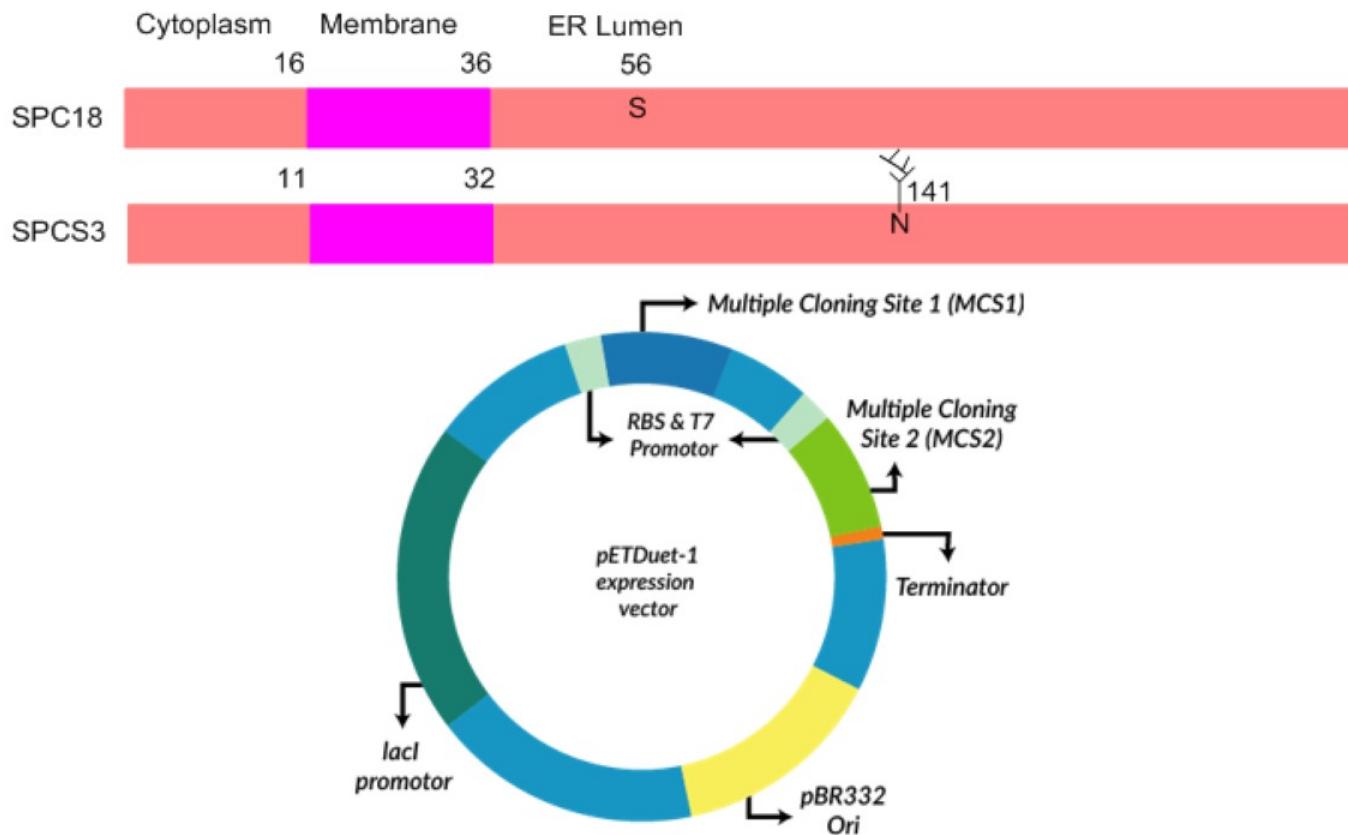
ER SPase I

	Human SPC18	Dog SPC18	Rat SPC18	Mouse SPC18	Rat SPC21	Mouse SPC21	Human SPC21	Dog SPC21	Drosophila melanogaster	Caenorhabditis elegans	Saccharomyces cerevisiae SEC11	Schizosaccharomyces pombe SEC11	Consensus
O75957	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S g S M E P a F H
P21378	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V L S G S M E P A F H	V R D I L f L t N r x
P42667													
Q9R0P6													
Q9WTR7													
Q9D8V7													
Q9BY50													
P13679													
O97066													
Q9N3D0													
P15367													
O74323													

	40	Box B	50	51	Box C	60	66	Box C'	75	81	Box D	93	94	Box E	112
Human SPC18	V L S G S M E P A F H	*			R G D L L F L T N R V		V G E I V V F R I E			I V H R V L K I H E K Q N	G H I K F L T K G D N N A V D D R G L				
Dog SPC18	V L S G S M E P A F H				R G D L L F L T N R V		V G E I V V F R I E			I V H R V L K I H E K Q N	G H I K F L T K G D N N A V D D R G L				
Rat SPC18	V L S G S M E P A F H				R G D L L F L T N R V		V G E I V V F R I E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Mouse SPC18	V L S G S M E P A F H				R G D L L F L T N R V		V G E I V V F R I E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Rat SPC21	V L S G S M E P A F H				R G D L L F L T N R V		V G E I V V F R I E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Mouse SPC21	V L S G S M E P A F H				R G D L L F L T N F R		A G E I V V F K V E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Human SPC21	V L S G S M E P A F H				R G D L L F L T N F R		A G E I V V F K V E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Dog SPC21	V L S G S M E P A F H				R G D L L F L T N F R		A G E I V V F K V E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Drosophila melanogaster	V L S G S M E P A F H				R G D L L F L T N F R		A G E I V V F K V E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Caenorhabditis elegans	V L S G S M E P A F H				R G D L L F L T N Y K		V G E I V V F K V E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Saccharomyces cerevisiae SEC11	V L S G S M E P A F H				R G D L L F L T N Y K		V G E I V V F K V E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Schizosaccharomyces pombe SEC11	V L S G S M E P A F H				R G D L L F L T N Y K		V G E I V V F K V E			I V H R V L K I H E K Q D	G H I K F L T K G D N N A V D D R G L				
Consensus	V L S g S M E P a F H	*			R G D I L f L t N r x		v G e i v V f k v e			I V H R V I K V H E K D N	G D I K F L T K G D N N E V D D R G L				

SPC18/SPCS3 co-expression

- Express ER Lumen only
- Express glycosylation by engineered *E. coli*



Paetzel, M., et al., *Chemical Reviews* 2002, 102(12), 4549-4580.

Shelness, G. S., et al., *Journal of Biological Chemistry* 1993, 268(7), 5201-5208.

Schwarz, F., et al., *Nat Chem Biol* 2010, 6(4), 264-266.

QikProp parameters

Property or Descriptor	Description	Range or recommended values	
molecule.name	Molecule name taken from the title line in the input structure file. If the title line is blank, the input file name is used.		0.0 – 0.13
#stars	Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. Outlying descriptors and predicted properties are denoted with asterisks (*) in the .out file. A large number of stars suggests that a molecule is less drug-like than molecules with few stars. The following properties and descriptors are included in the determination of #star: MW, dipole, IP, EA, SASA, FOSA, FISA, PISA, WPSA, PSA, volume, #rotor, donorHB, acptHB, glob, QPpolrz, QPlogPC16, QPlogPoct, QPlogPw, QPlogPo/w, logS, QPlogKhsa, QPlogBB, #metabol	0 – 5	0.0 – 0.05
amine	Number of non-conjugated amine groups.	0 – 1	0.75 – 0.95
guanidine	Number of amidine and guanidine groups.	0	13.0 – 70.0
acid	Number of carboxylic acid groups.	0 – 1	4.0 – 18.0
amide	Number of non-conjugated amide groups.	0 – 1	8.0 – 35.0
#rotor	Number of non-trivial (not CX3), non-hindered (not alkene, amide, small ring) rotatable bonds.	0 – 15	4.0 – 45.0
#rtvFG	Number of reactive functional groups; the specific groups are listed in the jobname.out file. The presence of these groups can lead to false positives in HTS assays and to decomposition, reactivity, or toxicity problems <i>in vivo</i> . See Appendix A of the <i>QikProp User Manual</i> for a complete list.	0 – 2	–2.0 – 6.5
CNS	Predicted central nervous system activity on a -2 (inactive) to +2 (active) scale.	-2 to +2	–6.5 – 0.5
mol_MW	Molecular weight of the molecule.	130.0 – 725.0	concern below -5
dipole†	Computed dipole moment of the molecule.	1.0 – 12.5	<25 poor, >500 great
SASA	Total solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 Å radius.	300.0 – 1000.0	–3.0 – 1.2
FOSA	Hydrophobic component of the SASA (saturated carbon and attached hydrogens).	0.0 – 750.0	<25 poor, >500 great
FISA	Hydrophilic component of the SASA (SASA on N, O, and H on heteroatoms).	7.0 – 330.0	–8.0 – –1.0
PISA	π (carbon and attached hydrogen) component of the SASA.	0.0 – 450.0	7.9 – 10.5
WPSA	Weakly polar component of the SASA (halogens, P, and S).	0.0 – 175.0	–0.9 – 1.7
volume	Total solvent-accessible volume in cubic angstroms using a probe with a 1.4 Å radius.	500.0 – 2000.0	1 – 8
donorHB	Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer.	0.0 – 6.0	
acptHB	Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer.	2.0 – 20.0	
RuleOffive	Number of violations of Lipinski's rule of five. The rules are: mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, acptHB ≤ 10. Compounds that satisfy these rules are considered drug-like. (The "five" refers to the limits, which are multiples of 5.)	maximum is 4	
RuleOfThree	Number of violations of Jorgensen's rule of three. The three rules are: , QPlogS > -5.7, QPlogKp > 22 nm/sec, # Primary Metabolites < 7. Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available.	maximum is 3	
#ringatoms	Number of atoms in rings.		>80% is high <25% is poor
#in34	Number of atoms in 3- or 4-membered rings.		0.0 – 100.0
#in56	Number of atoms in 5- or 6-membered rings.		0.0 – 35.0
#noncon	number of ring atoms not able to form conjugated aromatic systems (e.g. sp ³ C).		7.0 – 200.0
#nonHatr	Number of heavy atoms (nonhydrogen atoms).		2 – 15
Jm	Predicted maximum transdermal transport rate, $K_p \times MW \times S$ ($\mu\text{g cm}^{-2} \text{hr}^{-1}$). K_p and S are obtained from the aqueous solubility and skin permeability, QPlogKp and QPlogS. This property is only written to the output file; it is not used in any other calculations.		
	dip ² /V†	Square of the dipole moment divided by the molecular volume. This is the key term in the Kirkwood-Onsager equation for the free energy of solvation of a dipole with volume V.	
	ACxDN[†],5/SA	Index of cohesive interaction in solids. This term represents the relationship $(\text{acceptHB}(\text{,donorHB})) / (\text{SA})$; see <i>Bioorg. Med. Chem. Lett.</i> 2000 , <i>10</i> , 1155.	
	glob	Globularity descriptor, $(4\pi r^2) / (\text{ASA})$, where r is the radius of a sphere with a volume equal to the molecular volume. Globularity is 1.0 for a spherical molecule.	
	QPpolrz	Predicted polarizability in cubic angstroms.	
	QPlogPC16	Predicted hexadecane/gas partition coefficient.	
	QPlogPoct	Predicted octanol/gas partition coefficient.	
	QPlogPw	Predicted water/gas partition coefficient.	
	QPlogPo/w	Predicted octanol/water partition coefficient.	
	QPlogS	Predicted aqueous solubility, $\log S$. S in mol dm ⁻³ is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.	
	CIQPlogS	Conformation-independent predicted aqueous solubility, $\log S$. S in mol dm ⁻³ is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.	
	QPlogHERG	Predicted IC ₅₀ value for blockage of HERG K ⁺ channels.	
	QPlogCaco	Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut-blood barrier. QikProp predictions are for non-active transport.	
	QPlogBB	Predicted brain/blood partition coefficient. Note: QikProp predictions are for orally delivered drugs so, for example, dopamine and serotonin are CNS negative because they are too polar to cross the blood-brain barrier	
	QPMDCK	Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier. QikProp predictions are for non-active transport.	
	QPlogKp	Predicted skin permeability, $\log K_p$.	
	IP(ev)†	PM3 calculated ionization potential.	
	EA(eV)†	PM3 calculated electron affinity.	
	#metab‡	Number of likely metabolic reactions. See Appendix A of the <i>QikProp User Manual</i> for a complete list of reactions.	
	QPlogKhsa	Prediction of binding to human serum albumin.	
	HumanOralAbsorption	Predicted qualitative human oral absorption: 1, 2, or 3 for low, medium, or high. The text version is reported in the output. The assessment uses a knowledge-based set of rules, including checking for suitable values of PercentHumanOralAbsorption, number of metabolites, number of rotatable bonds, logP, solubility and cell permeability.	
	PercentHumanOralAbsorption	Predicted human oral absorption on 0 to 100% scale. The prediction is based on a quantitative multiple linear regression model. This property usually correlates well with HumanOralAbsorption, as both measure the same property.	
	SAFluorine	Solvent-accessible surface area of fluorine atoms.	
	SAamideO	Solvent-accessible surface area of amide oxygen atoms.	
	PSA	Van der Waals surface area of polar nitrogen and oxygen atoms.	
	#NandO	Number of nitrogen and oxygen atoms.	

Original Arylomycin A2 crystal structure

- PDB – 1T7D

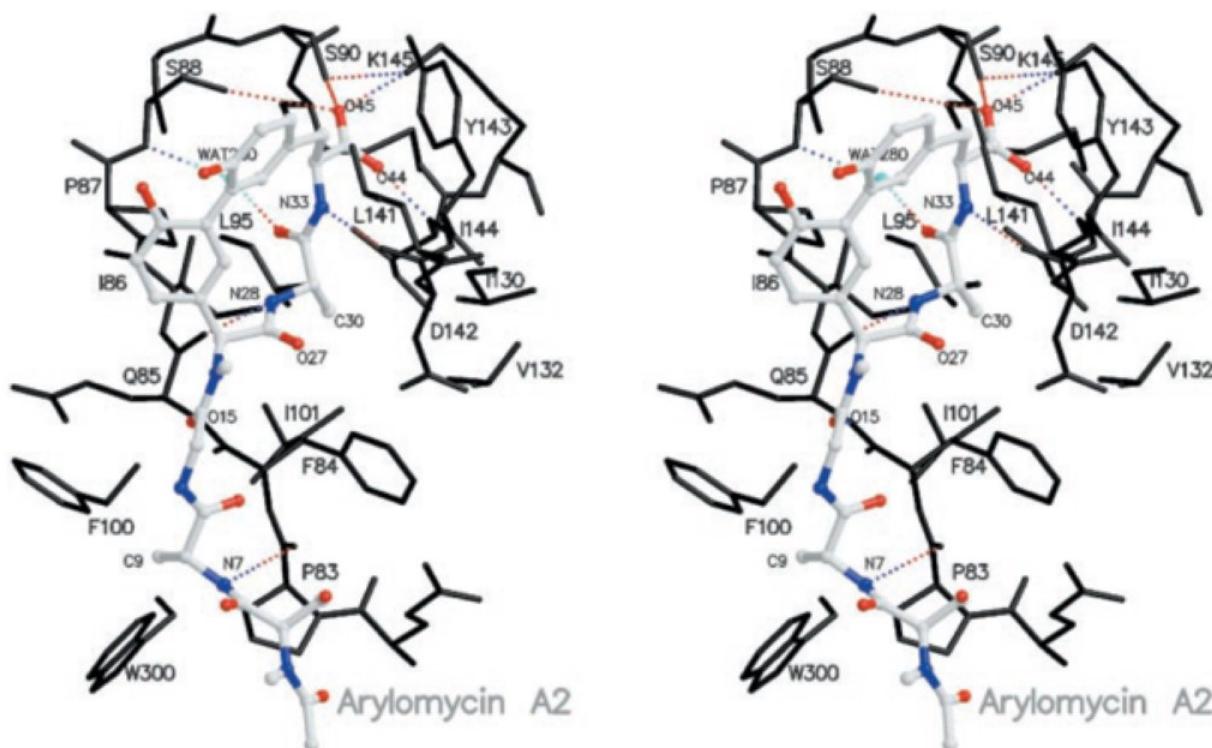
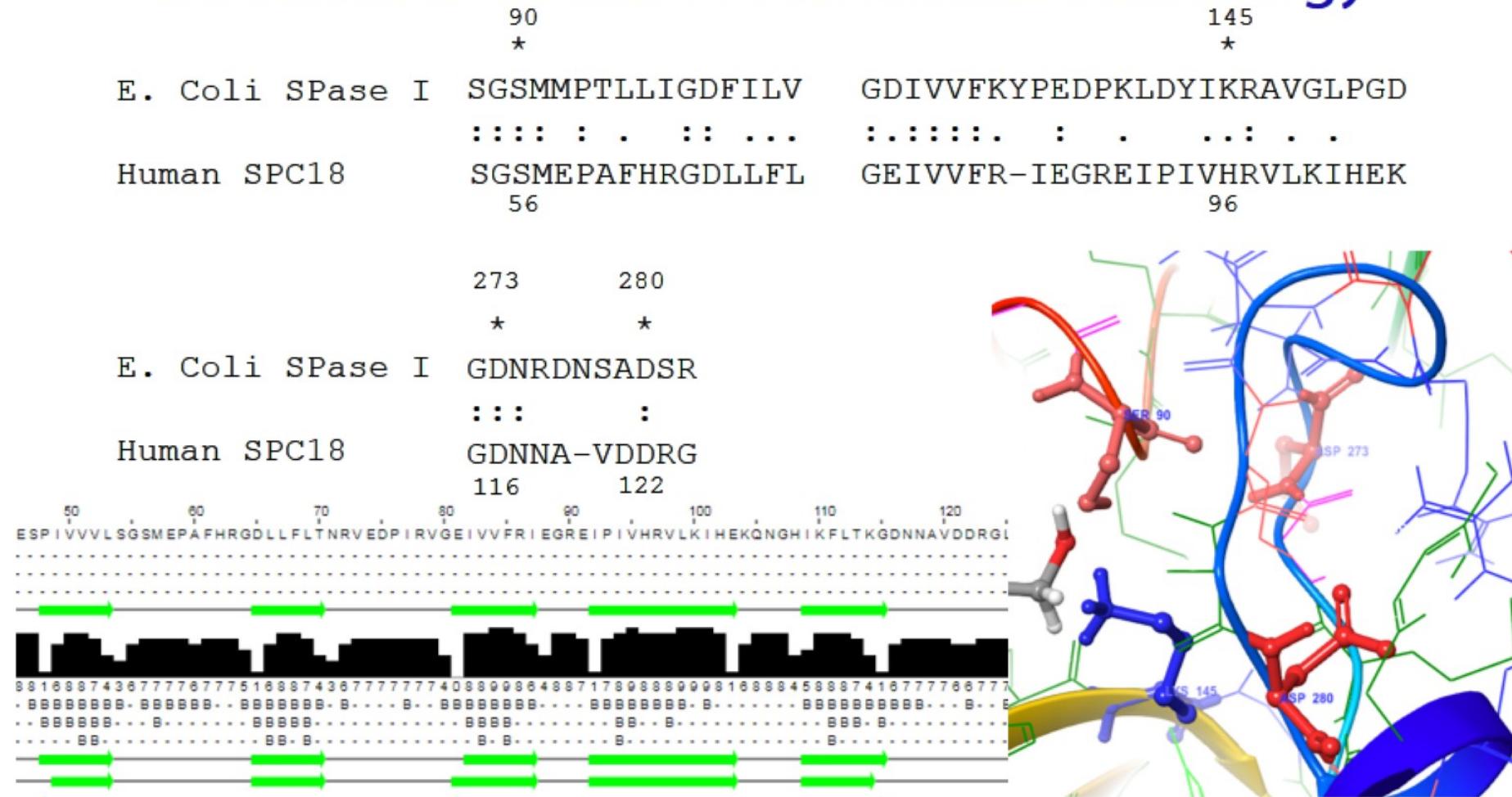


FIG. 3. Structure of the active site of signal peptidase with a noncovalently bound biaryl-bridged lipohexapeptide inhibitor. A stereo rendering of arylomycin A₂ bound in the active site of *E. coli* type I SPase. The protein is in *black stick* and the inhibitor is in ball-and-stick with *gray* for carbon, *blue* for nitrogen, and *red* for oxygen.

Active site sequence homology

- Residues near active site shows homology



Qikprop log of 1

QikProp v5.1 (rel 11) - Properties Predictions for
1

Primary Metabolites & Reactive FGs:

- > Metabolism likely: allylic H → alcohol
- > Metabolism likely: allylic H → alcohol
- > Metabolism likely: alpha, beta dehydrogenation at carbonyl
- > Metabolism likely: alpha, beta dehydrogenation at carbonyl
- > Metabolism likely: secondary alcohol → ketone
- > Metabolism likely: alpha, beta dehydrogenation at carbonyl
- > Metabolism likely: alpha, beta dehydrogenation at carbonyl
- > Metabolism likely: alpha, beta dehydrogenation at carbonyl
- > Metabolism likely: alpha, beta dehydrogenation at carbonyl
- > Reactive FG: unhindered ester
- > Metabolism likely: alpha, beta dehydrogenation at carbonyl

Principal Descriptors:

		(Range 95% of Drugs)
Solute	Molecular weight	= 792.067 (130.0 / 725.0)*
Solute	Dipole Moment (D)	= 5.767 (1.0 / 12.5)
Solute	Total SASA	= 1406.800 (300.0 / 1000.0)*
Solute	Hydrophobic SASA	= 1155.340 (0.0 / 750.0)*
Solute	Hydrophilic SASA	= 237.527 (7.0 / 330.0)
Solute	Carbon Pi SASA	= 13.933 (0.0 / 450.0)
Solute	Weakly Polar SASA	= 0.000 (0.0 / 175.0)
Solute	Molecular Volume (A^3)	= 2681.822 (500.0 / 2000.0)*
Solute	vdw Polar SA (PSA)	= 238.895 (7.0 / 200.0)*
Solute	No. of Rotatable Bonds	= 30.000 (0.0 / 15.0)*
Solute as Donor -	Hydrogen Bonds	= 1.000 (0.0 / 6.0)
Solute as Acceptor -	Hydrogen Bonds	= 14.700 (2.0 / 20.0)
Solute Globularity (Sphere = 1)		= 0.664 (0.75 / 0.95)*
Solute Ionization Potential (ev)		= 9.346 (7.9 / 10.5)
Solute Electron Affinity (ev)		= -0.101 (-0.9 / 1.7)

Predictions for Properties:

QP Polarizability (Angstroms^3)	= 82.043M (13.0 / 70.0)*
QP log P for hexadecane/gas	= 27.046M (4.0 / 18.0)*
QP log P for octanol/gas	= 36.272M (8.0 / 35.0)*
QP log P for water/gas	= 20.578M (4.0 / 45.0)
QP log P for octanol/water	= 5.040 (-2.0 / 6.5)
QP log S for aqueous solubility	= -8.059M (-6.5 / 0.5)*
QP log S - conformation independent	= -6.161M (-6.5 / 0.5)
QP Log K hsa Serum Protein Binding	= -0.143M (-1.5 / 1.5)
QP log BB for brain/blood	= -4.841M (-3.0 / 1.2)*
No. of Primary Metabolites	= 9 (1.0 / 8.0)*
Predicted CNS Activity (-- to ++)	= --
HERG K+ Channel Blockage: log IC50	= -0.170M (concern below -5)
Apparent Caco-2 Permeability (nm/sec)	= 11M (<25 poor, >500 great)
Apparent MDCK Permeability (nm/sec)	= 21M (<25 poor, >500 great)
QP log Kp for skin permeability	= -2.968M (Kp in cm/hr)
Jm, max transdermal transport rate	= 0.000M (micrograms/cm^2-hr)
Lipinski Rule of 5 Violations	= 3 (maximum is 4)
Jorgensen Rule of 3 Violations	= 3 (maximum is 3)
% Human Oral Absorption in GI (+-20%)	= 36M (<25% is poor)
Qual. Model for Human Oral Absorption	= lowM (>80% is high)

molecule	CIQPlogS	QPlogBB
1	-6.161	-4.841
10	-3.036	-3.481
17	-4.426	-3.04
18	-3.717	-3.276
22	-5.107	-3.03
26	-3.496	-3.36

A * indicates a violation of the 95% range. # stars = 13
An M indicates MW is outside training range.

Log file of docking for 9

```
[running at reduced cpu priority]
vdw radii of ligand atoms scaled by 0.8000000000000000
charge cutoff for polarity 0.1500000000000000

GLIDE CONSTRAINTS APPLIED
-----  
2 out of 2 constraints used from file C:\users\Administrator\Documents\Schrodinger\glide-grid_22 (default)\glide-grid_22.cons
Labels and receptor types of constraints used are:
position1 (Positional)
position2 (Positional)

All docked poses must satisfy the specified number of
constraints in ALL of the following 1 groups.
Group 1: At least 2 of (position1, position2)

After readscreen, (nx, ny, nz) = ( 41 , 41 , 41 ).  
Receptor setup: (nsites, nx, ny, nz, bsize)=( 125 , 41 , 41 , 41 , 1.0000000000000000 ).  
Screening setup finished.  
DOCKMAIN: getting receptor.  
DOCKMAIN after grid: (nx, ny, nz) = ( 112 , 112 , 112 ).  
DOCKMAIN: Grid setup finished  
Calling OPLS3 atomtyping ...  
Finished parameter assignment

Penalizing non-planar amide torsions

Number of rotatable bonds 21
Using templates for ring conformations.

GlideScore version SP5.0 will be used

Buried polar penalty 0.000
Coulomb vdw cutoff 30.000
H bond cutoff 0.000
Metal-ligand cutoff 10.000
Assigning GlideScore SP5.0 parameters
Checked constraints after refinement with max HB dsq = 16.00000000000000
After refinement, constraint filter reduced number of
poses from 2805 to 459 .
Total constraint copies over all poses = 489
TOO MANY WATER SITES 2000001
Postdocking minimization: 5 poses; CvDW cutoffs 130.0 kcal/mol for min, 30.0 kcal/mol for report.
Constraint(s) matched for ligand 1, pose 168
Score = -5.702
Emodel = -70.394

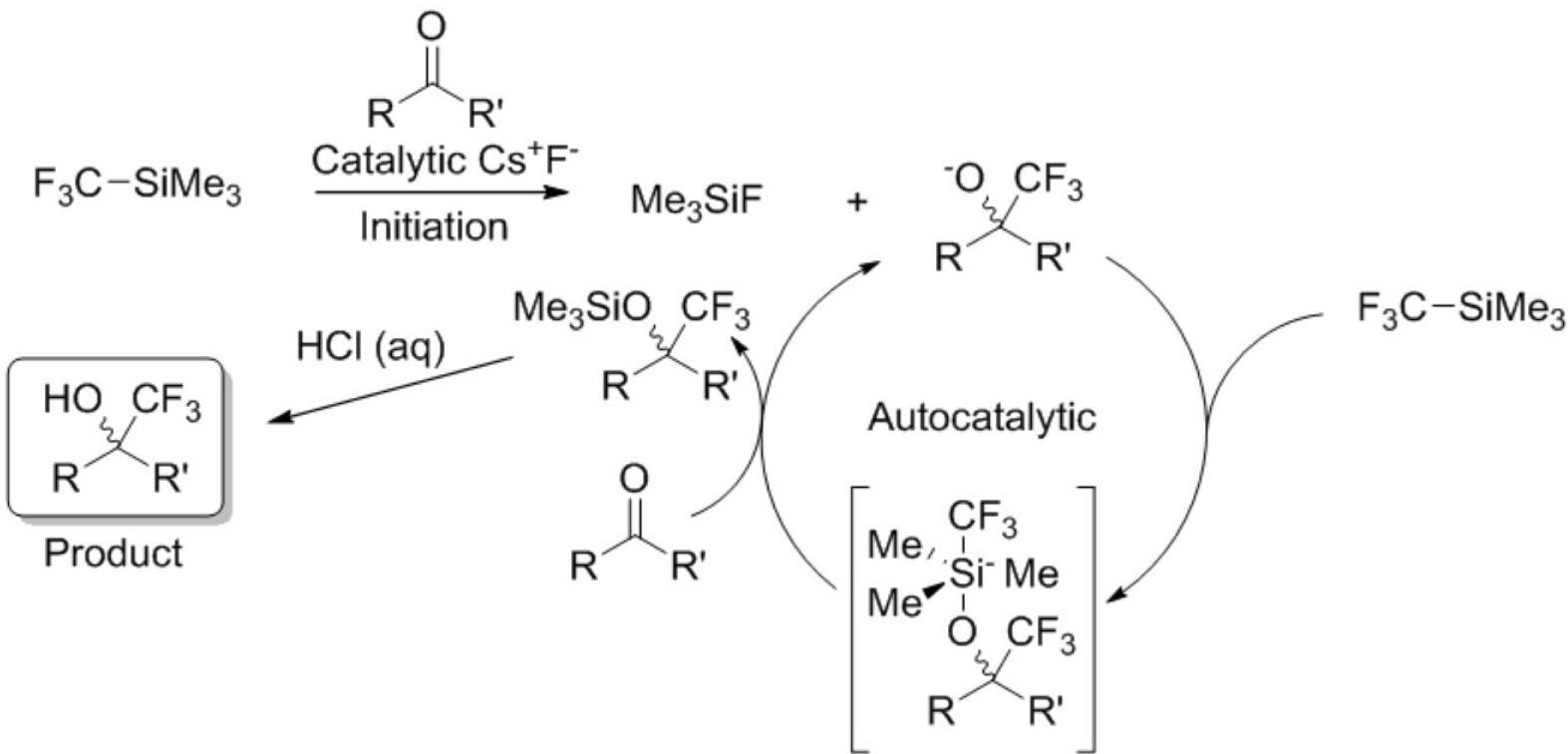
The output pose satisfies at least the following constraint(s).
position1 (Type = Positional)
position2 (Type = Positional)

GlideScore( -5.70)+Epik State Penalty( 0.00) = -5.70 kcal/mol
DOCKING RESULTS FOR LIGAND 1 (X9)
Except for Best Emodel, the poses reported here
may not satisfy the user-specified constraints.
Best Emodel= -70.39 E= -54.33 Eint= 11.53 GlideScore= -5.70

REPORT OF DOCKED POSES

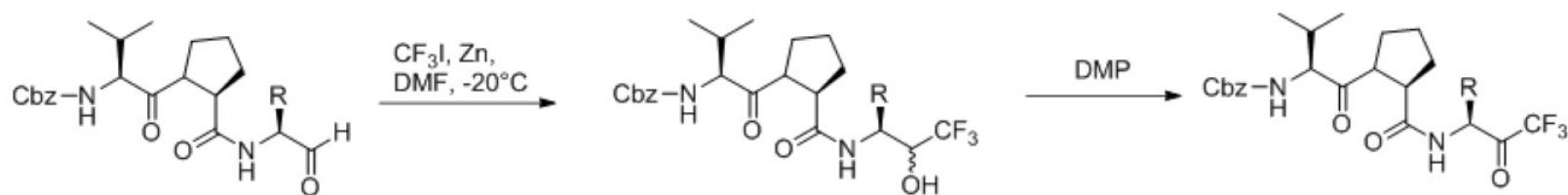
The ligand structures were written to the file
glide-dock_SP_40-0001_raw.maegz
```

TMSCF₃ mechanism



Alternative trimethylfluorination method

- Stereoselective (>90%)
- DMP will not racemize



Inhibitor kinetics

$$IC_{50} = K_i \left(1 + \frac{[S]}{K_M} \right)$$

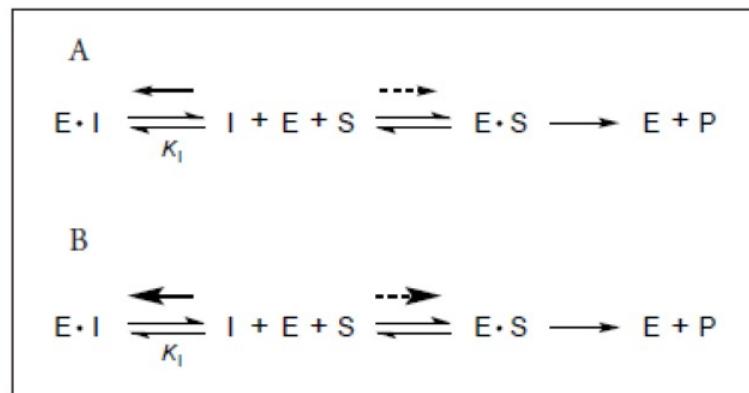


Figure 1. Competitive inhibition, A: IC_{50} determined at low $[S]$, B: IC_{50} determined at high $[S]$.

Capecitabine

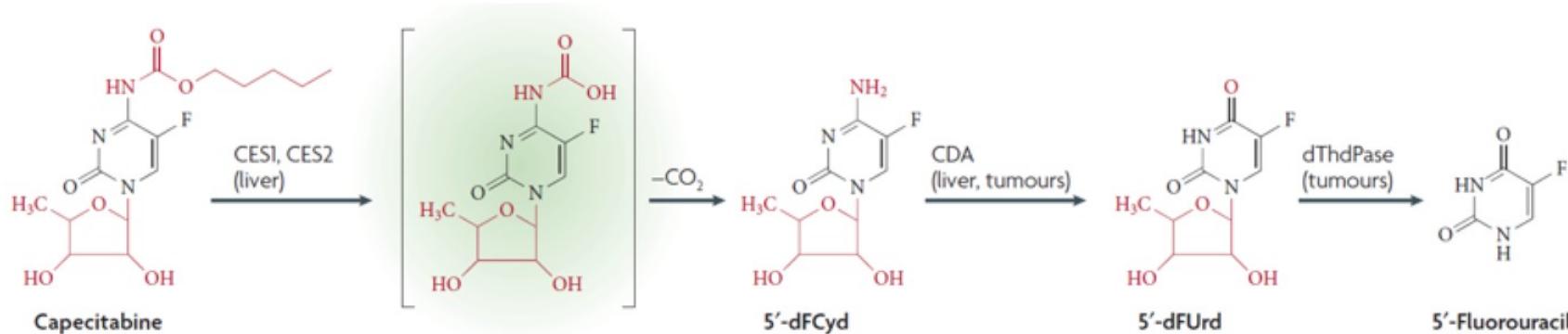
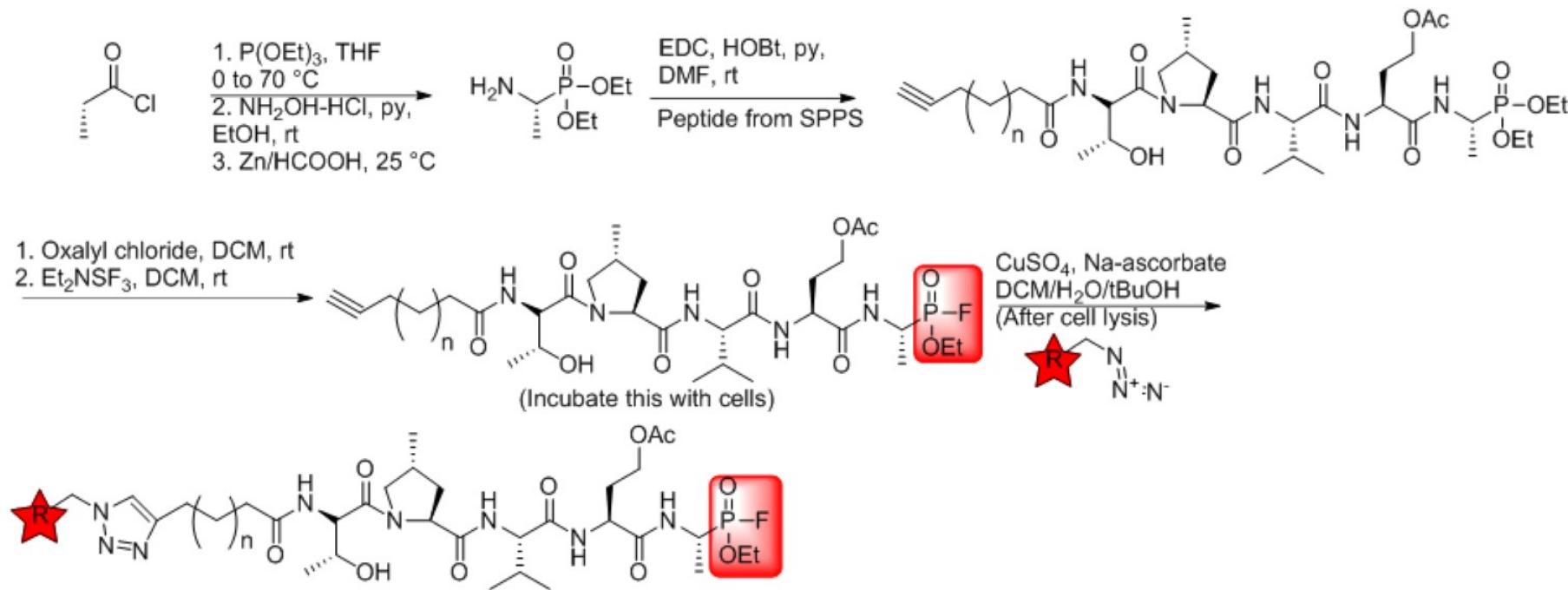


Figure 3 | Capecitabine as an example of a prodrug that requires multiple enzymatic activation steps.
Capecitabine (Xeloda) is a prodrug that has reduced gastrointestinal toxicity and high tumour selectivity.

The enzymatic bioconversion pathway initiates in the liver, where human carboxylesterases 1 and 2 (CES1 and CES2) cleave the ester bond of the carbamate¹⁴². This is followed by a fast, spontaneous decarboxylation reaction resulting in 5'-deoxy-5-fluorocytidine (5'-dFCyd)¹⁴⁴. Generation of the parent drug continues in the liver, and to some extent in tumours, by cytidine deaminase (CDA), which converts 5'-dFCyd to 5'-deoxyuridine (5'-dFUr). Finally, thymidine phosphorylase (dThdPase; also known as ECGF1) liberates the active drug 5'-fluorouracil in tumours^{7,144}.

Synthesis of ABPs



Yang, S. H., et al., *Organic Letters* 2011, 13(20), 5604-5607.

Wang, Q., et al., *Journal of the American Chemical Society* 2003, 125(11), 3192-3193.

Gillet, L. C. J., et al., *Molecular & Cellular Proteomics* 2008, 7(7), 1241-1253.

Ntai, I., et al., *Bioorganic & Medicinal Chemistry Letters* 2008, 18(10), 3068-3071.