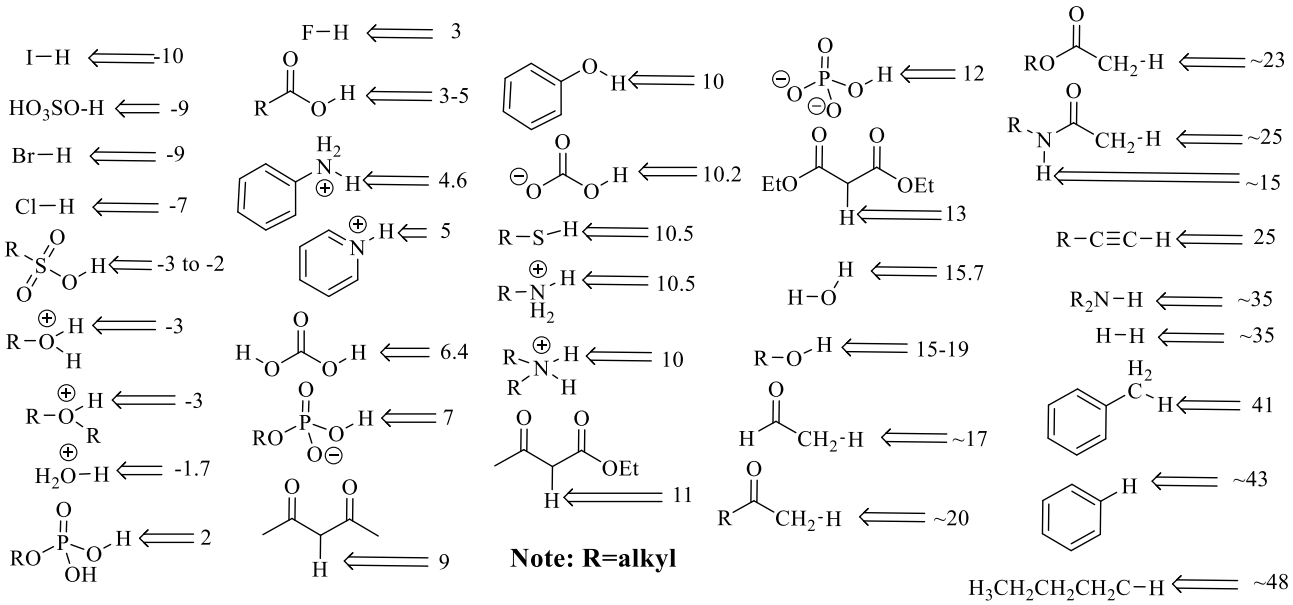
Medicinal Chemistry Name: Dipa Kalyani
CHEM 515
Final Exam
Tuesday, May 6, 2025

Read each question carefully before answering. Be certain you understand everything the question is requesting. Do the easy questions first. If questions appear confusing or exceedingly complex, then you may need to rethink the question. Keep in mind the intended examination topics.

In medicinal chemistry, hand-drawn pictures convey specific information. Be sure the drawing you have made conveys the essential information required to answer the question. Make certain that three-dimensional pictures display the correct atom arrangements. Don't forget to include formal charges when appropriate.

The exam is worth a total of 150 points. It has 15 fifteen-point questions on the exam and you need to answer at least 10 of the 15 questions. If you answer more than 10 questions, I will only count your 10 answers with the most points. *Bonus points <u>will</u> be awarded for your eleventh additional correct answer.*

<u>pK_a information</u>

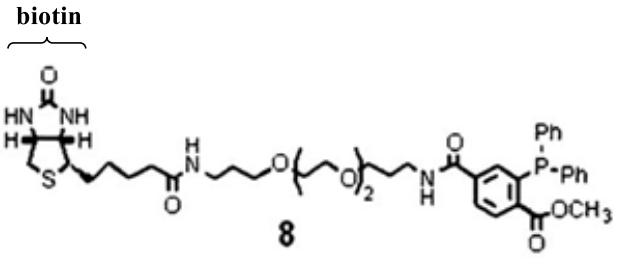


1. "A chemical approach for identifying O-GlcNAc-modified proteins in cells" Proceedings of the National Academy of Sciences 2003, Vol. 100, pp. 9116-9121. C. Bertozzi et al. *(Carmen and Juanita, March 18)*

a) Prof. Bertozzi and co-workers described some impressive bio-orthogonal innovations that lead to their Nobel Prize award in 2022. In your own words, explain the tool that they created, how it worked and the overall goal of the project. (5 pts.)
(https://www.pnas.org/doi/10.1073/pnas.1632821100)

Prof. Bertozzi and her fellow researchers created modified sugars (GlcNAz) with an azido group attached to the acetyl piece of an N-acetyl-glucosamine sugar. They were able to introduce that azide-modified sugar into a cell system and get it incorporated into proteins at serine and threonine amino acid side chains. The hexosamine salvage pathway was exploited/hijacked to incorporate GlcNAz into the cells and make UDP-GlcNAz molecules that were then attached to Ser or Thr side chains of various proteins. The researchers demonstrated that various proteins were labeled with the GlcNAz group and that the proteins could be modified through a Staudinger reaction with a phosphine group. This process allowed the identification of the proteins that get glycosylated and the site of the glycosylation events.

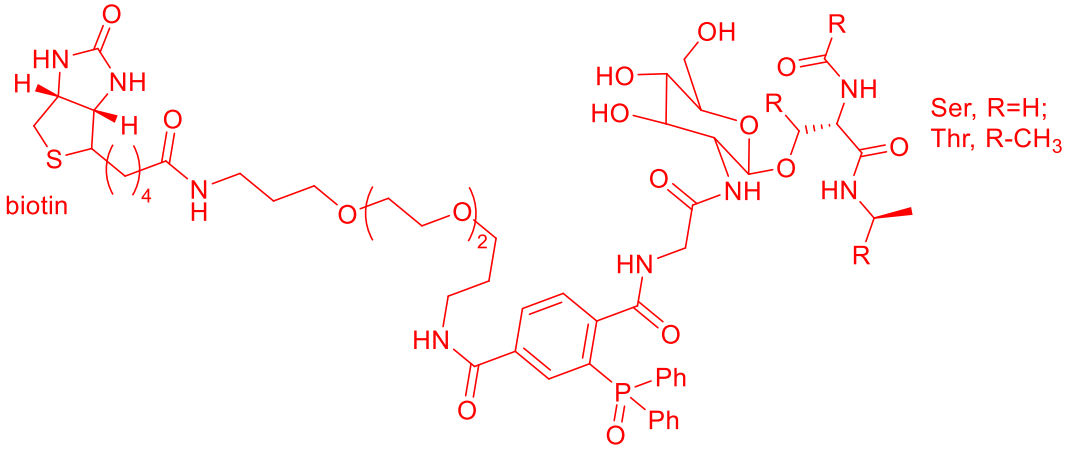
Answer the following questions about compound **8**, which was reported by Prof. Bertozzi and her co-workers in the 2003 PNAS article that was discussed by Carmen and Juanita on March 18.



b) Show the final product of the reaction of compound **8** with an O-GlcNAz saccharide (GlcNAz= N-2-azidoacetyl-glucosamine). Your picture should include items **i** and **ii** below. You may abbreviate the structures, but be sure key fragments are shown to address the requests below. (6 pts.)

(i) the amino acid side chain to which the O-GlcNAz is connected. Draw the amino acid side chain with a small segment of the peptide backbone shown.

(ii) the final bonding arrangement or structure of the phosphine group and the carboxylic ester group.

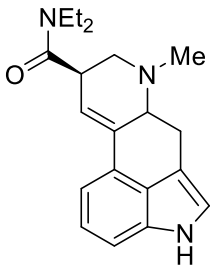


c) After compound **8** is introduced to and reacts with the cell proteins with O-GlcNAz incorporated, the following direction is offered: "Membranes were then incubated with a solution of blocking buffer A containing streptavidin-horseradish peroxidase (HRP) conjugate or blocking buffer B containing anti-FLAG-HRP conjugate." What is reacting with compound **8**, where is it reacting, what role does the HRP play? (4 pts.)

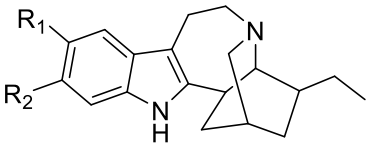
The biotin moiety of compound **8** binds with incredibly high affinity (K_d~ 10^{-15} M) to streptavidin, and, as described in the experimental description, horseradish peroxidase (HRP) was attached to the streptavidin. Therefore, the biotin-streptavidin connection allowed the attachment of the horseradish peroxidase (HRP) to the O-GlcNAz via the phosphine group and a Staudinger reaction. Once connected, a chemical reagent (including luminol) was added to the complex and HRP caused an oxidation reaction of the chemical reagent that creates a visible stain on the gel and allows detection of the O-GlcNAz saccharides.

2. "A non-hallucinogenic psychedelic analogue with therapeutic potential", Nature 2021, Vol. 589, pp. 474–479, D. E. Olson et al. *(Chi and Sahana, March 20)*

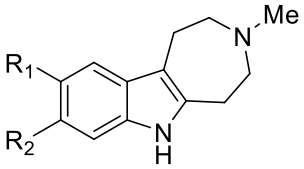
a) Briefly explain what a pharmacophore is and then identify the common pharmacophore for the following neuroactive agents by circling and/or drawing it. (5 pts.)



lysergic acid diethylamide (LSD)

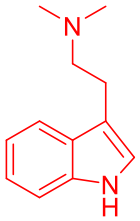


ibogaine



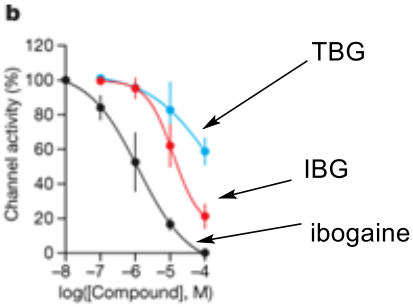
tabernanthalog (TBG)

pharmacophore



A pharmacophore is the key structural element in a molecule or set of molecules that is responsible for the biological effect or therapeutic activity.

b) Figure 2b is reproduced below. It shows an analysis of hERG (human ether-a-go-go) channel activity. Why were the researchers concerned with hERG channels and which compound showed the least amount of inhibition? Approximate the IC_{50} values of each to the nearest tens digit in molarity, M. (4 pts.)

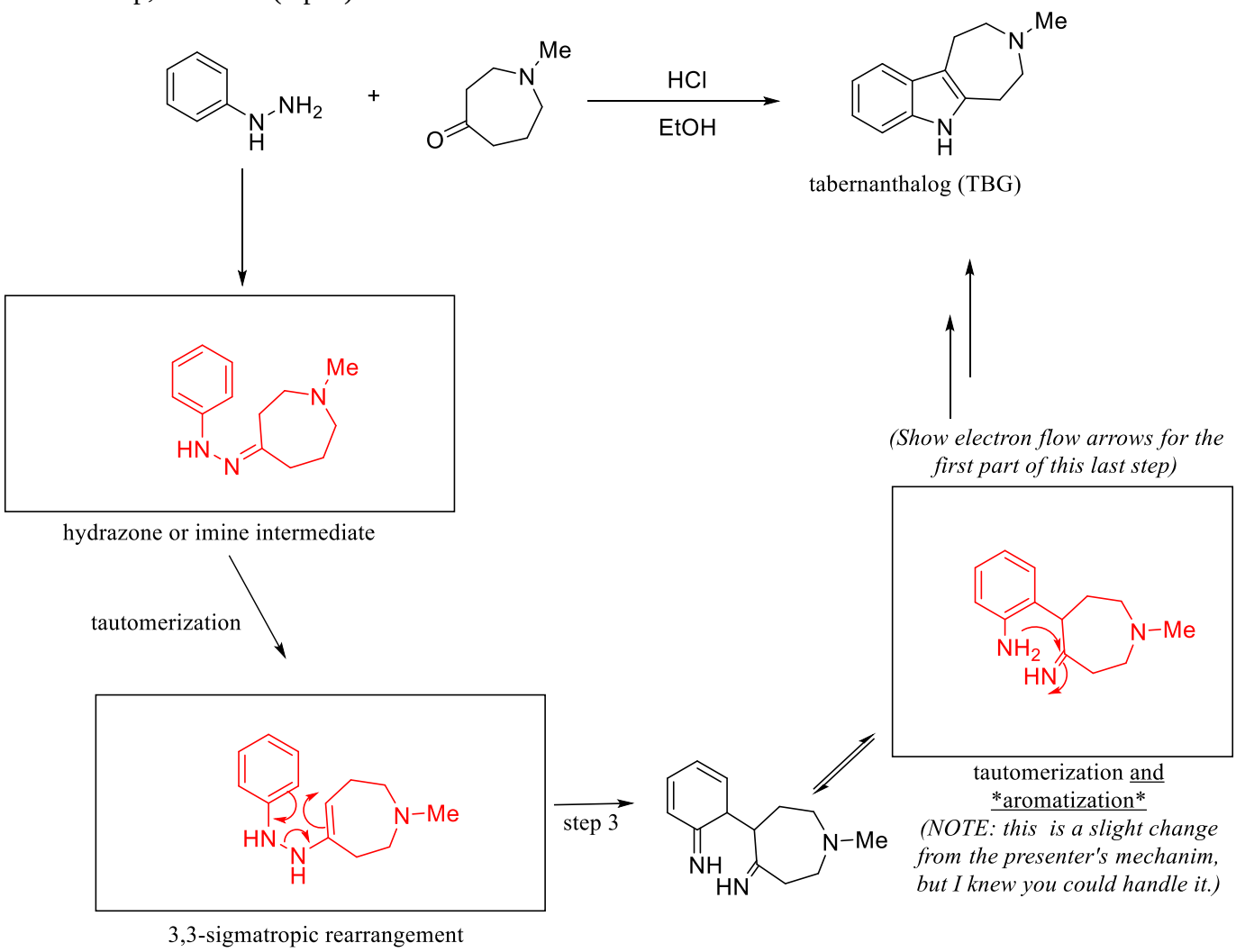


Legend text: Inhibition of hERG channels expressed in HEK293 cells. Data are mean ± s.d. IBO, ibogaine (black); IBG, ibogainalog (red); TBG, tabernanthalog (blue)

hERG (human ether-a-go-go) is a receptor in the heart and inhibition of it can cause serious cardiac problems or cardiotoxicity. All drugs need to be screened for hERG activity.

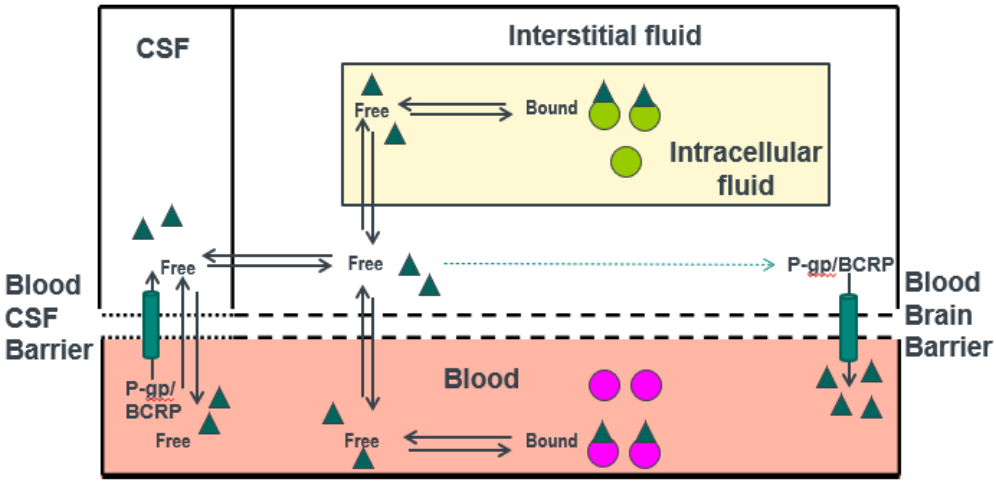
TBG showed the least hERG inhibition potency at roughly ~10^{-3} or 10^{-4} M, while IBG was next at ~10^{-5} M and IBO showed the most potent inhibition at ~10^{-6} M.

c) Shown below is the key reaction to form the TBG drug structure. Complete the sequence by showing the intermediates and providing the curved electron flow arrows for the 3,3-sigmatropic rearrangement (step 3) and the final step, as noted. (5 pts.)



3. Dr. Kelly Bleasby, Bristol Myers Squibb, March 25: "Optimizing Blood-Brain-Barrier Penetration: Considerations for Drug Design, Delivery and Translation". *Lecture reference*: N. C. Patel et al. Harnessing Preclinical Data as a Predictive Tool for Human Brain Tissue Targeting | ACS Chemical Neuroscience, *ACS Chem. Neurosci.* **2021**, *12*, 6, 1007-1017

In Dr. Bleasby's presentation of "Optimizing Blood-Brain-Barrier Penetration: Considerations for Drug Design, Delivery and Translation", she presented the following diagram. Please answer the following questions about the diagram and based on the topics discussed.



a) What's all the 'free' stuff about, i.e. the triangles? (2 pts.)
The triangles and 'free' represent free drug that isn't bound to plasma proteins.

b) Why are some of the triangles attached to circles? What does that represent? (3 pts.)
The circles are plasma proteins that can bind the drug and prevent the drug's free circulation and association with the target biomolecule.

c) What is P-gp and BCRP, and what are they doing in the brain and the CSF? (3 pts.)
P-gp is P-glycoprotein and BCRP is the breast cancer resistance protein. Both are transporter proteins that facilitate drug or other molecules crossing various cellular barriers. In the blood brain barrier (BBB), they efflux drugs or molecules out of the brain and into the blood/capillaries. In the choroid plexus, they influx drugs/molecules into the cerebral spinal fluid (CSF) from the blood/capillaries.

d) What is the relationship between the triangle concentration in the CSF versus the brain? (4 pts.)
The triangles or drugs passively diffuse from the cerebral spinal fluid (CSF) into the brain. If there are high levels of passive diffusion of a drug/molecule, then the concentrations of a drug/molecule between the CSF and the brain will be equal. If, on the other hand, there are active efflux transporter proteins, then the concentration of the drug in the CSF will typically be greater than in the brain.

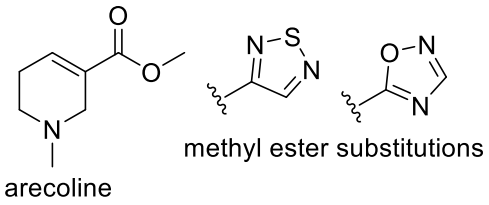
e) For a drug that is designed to interact with a target in the brain, what will determine the pharmacological or therapeutic response? (3 pts.)
The amount or concentration of free drug in the brain will determine the amount of drug that can interact with the target and elicit the desired response.

4. "Classics in Chemical Neuroscience: Xanomeline" ACS Chemical Neuroscience, 2017, Vol. 8, pp. 435-443. Craig W. Lindsley et al. *(Stirling and Gabriela, March 27)*
a) What is the mechanism of action of xanomeline and how is this thought to benefit treatment of Alzheimer's disease and schizophrenia? (3 pts.)

Xanomeline is a muscarinic acetylcholine receptor agonist, which means upon binding at a receptor it will elicit a biological effect consistent with acetylcholine binding at the same receptor. Decreased acetylcholine activity is believed to be the cause of Alzheimer's disease, schizophrenia, and other neurological disorders (the cholinergic hypothesis of neurodegenration).

b) Arecoline was the natural product inspiration for xanomeline. Two methyl ester substitutions made to the arecoline lead structure are shown.

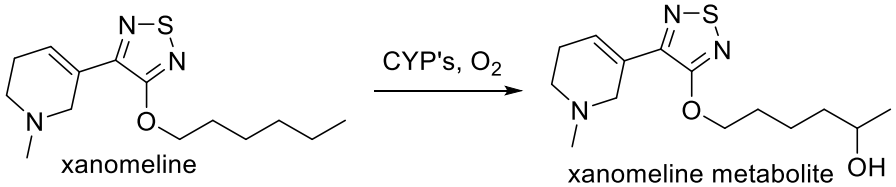
(i) In medicinal chemistry, what is the term used for these types of changes to a particular functional group? (2 pts.)
These represent "isosteric changes" as the groups have similar shape and electronics as the ester.

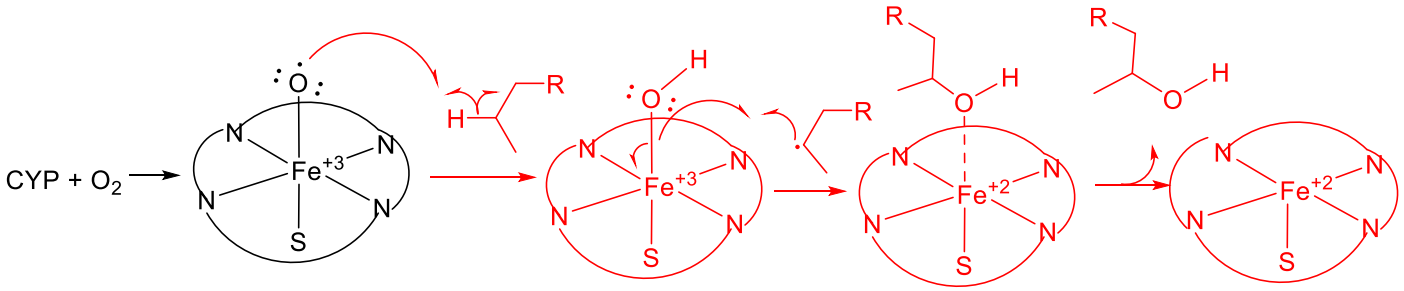


(ii) List two benefits that might be realized by changing the methyl ester to either of the heteroaromatic rings shown. (2 pts.)

The heteroaromatic rings would likely be more stable to hydrolysis or esterases and they would enhance solubility by introducing more polar heteroatoms capable of hydrogen bonding.

c) One of xanomeline's metabolic products as reported in the article is shown below. Draw the mechanism of cytochrome P-450's (CYP's) reaction with xanomeline to cause the metabolite formation. You are encouraged to abbreviate xanomeline's structure to only show the relevant alkyl chain piece in drawing your mechanism. (5 pts.)





d) As represented in the metabolic reaction shown in part **c**, what is the body's general goal in conducting metabolic reactions with drugs? How is this occurring with the xanomeline metabolite? (3 pts.)
The body is trying to polarize drugs or any foreign substances to allow their rapid removal or excretion from the body through promoting their ability to dissolve in aqueous solutions. By adding a hydroxyl group to the alkyl chain of xanomeline, the metabolite is now more polar and would be more soluble in water.

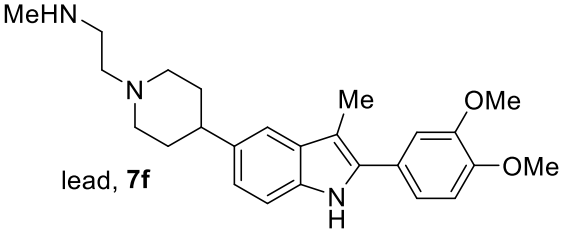
5. Dr. Trevor Sherwood, Bristol Myers Squibb, April 1: "Discovery of afimetoran, a first-in-class small molecule dual antagonist of TLR7/8 for the treatment of lupus". *Lecture reference*: A. J. Dyckman et al. Identification of 2-Pyridinylindole-Based Dual Antagonists of Toll-like Receptors 7 and 8 (TLR7/8) | ACS Medicinal Chemistry Letters, *ACS Med. Chem. Lett.* **2022**, 13, 5, 812–818
In Dr. Sherwood's presentation, he noted that one of the problems with the lead compound (compound **7f** below) was the high V_{ss}, or the apparent volume distribution of the drug at steady state. As the second article Dr. Sherwood shared (ACS Med. Chem. Lett. 2022, 13, 812−818) states, "While the observed compound blood clearance was high (Cl = 146 (mL/min)/kg), the blood volume of distribution was also quite high (V_{ss} = 113 L/kg) contributing to the observed long terminal $T_{1/2}$."
a) What is the V_{ss} or, the related V_d (volume of distribution), and why does a high value for V_{ss} or V_d undermine a drug's therapeutic effect? (5 pts.)

The V_d is defined as $V_d = A/C$, where A is the total amount of drug introduced into the body and C is the drug concentration in blood plasma. Therefore, too little drug in the plasma (small C value) results in a high V_d value and little drug actually circulating in the blood and available to interact with the desired target. With a high V_d, more of the drug is retained in other organs and areas of the body.

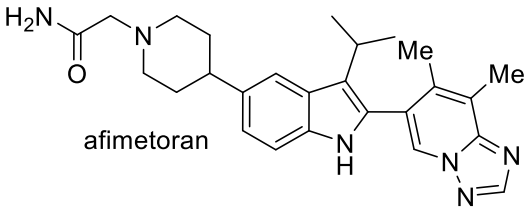
b) Why is it surprising that the drug clearance was high, but that the drug's V_{ss} was also high and why did that contribute to a long drug $T_{1/2}$? Briefly explain why these principles might be contradictory or reinforcing based on your understanding of V_{ss}, clearance (Cl) and $T_{1/2}$. Your explanation should include a brief description of clearance and $T_{1/2}$. (5 pts.)

Clearance is the amount of drug removed from a system over time. To have a high Cl, would suggest the drug is rapidly removed from the body or system and there is a low level of drug concentration in the body. However, in this case, much of the drug is retained in other regions of the body, based on the high V_d. The retention of the drug in organs and other regions of the body allows the drug to be slowly released into the system over a longer period of time leading to the longer drug half-life $T_{1/2}$. The half-life is the time that it takes to remove half of the drug from the system or body.

c) During his talk, Dr. Sherwood also explained modifications made to the N-alkylamine substituent on the piperidine ring, including the introduction of carbonyl groups on the alkyl chain to make the derivatives "monobasic, dibasic and nonbasic". In the final drug candidate, afimetoran (shown below), there is a carbonyl added to the N-alkylamine side chain. What impact might this have on the V_{ss} or related V_d of the drug and its ability to reach the TLR 7/8 target? (5 pts.)



lead, **7f**



afimetoran

The introduction of the carbonyl makes an amide group in afimetoran, which has much lower basicity than the alkyl amine of **7f**. As noted on the NIH NLM website, basic functional groups in drugs, like the amine of **7f**, contributes to the drug's distribution and retention by the anionic phospholipid head groups of cell membranes. This makes the drugs less available in the blood plasma and therefore reduces the drug's tendency to circulate through the body and reach the intended target, TLR 7/8. The amide of afimetoran is less basic so it would have a lower V_{ss} and remain available in the blood plasma to interact with the target protein.

6. "Synthesis and target annotation of the alkaloid GB18", Nature 2022, Vol. 606, pp. 917–921. S. Woo and R. A. Shenvi *(Alex and Sidney, April 3)*
a)

b) Figure 4a from the article is reproduced here. What were the researchers doing with GB18 in this assay and what did they discover? Your answer should address the items listed along the x-axis of the graph, what the y-axis is measuring and how that was accomplished. (5 pts.)

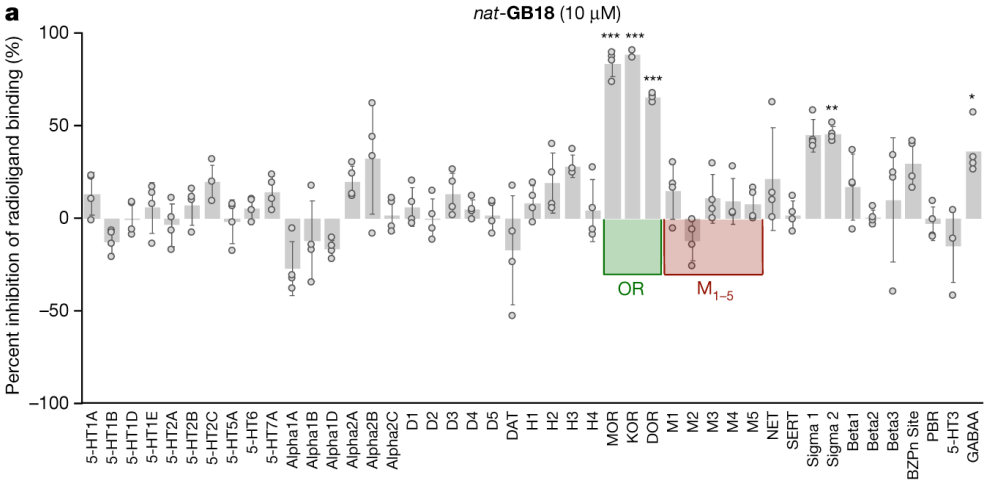
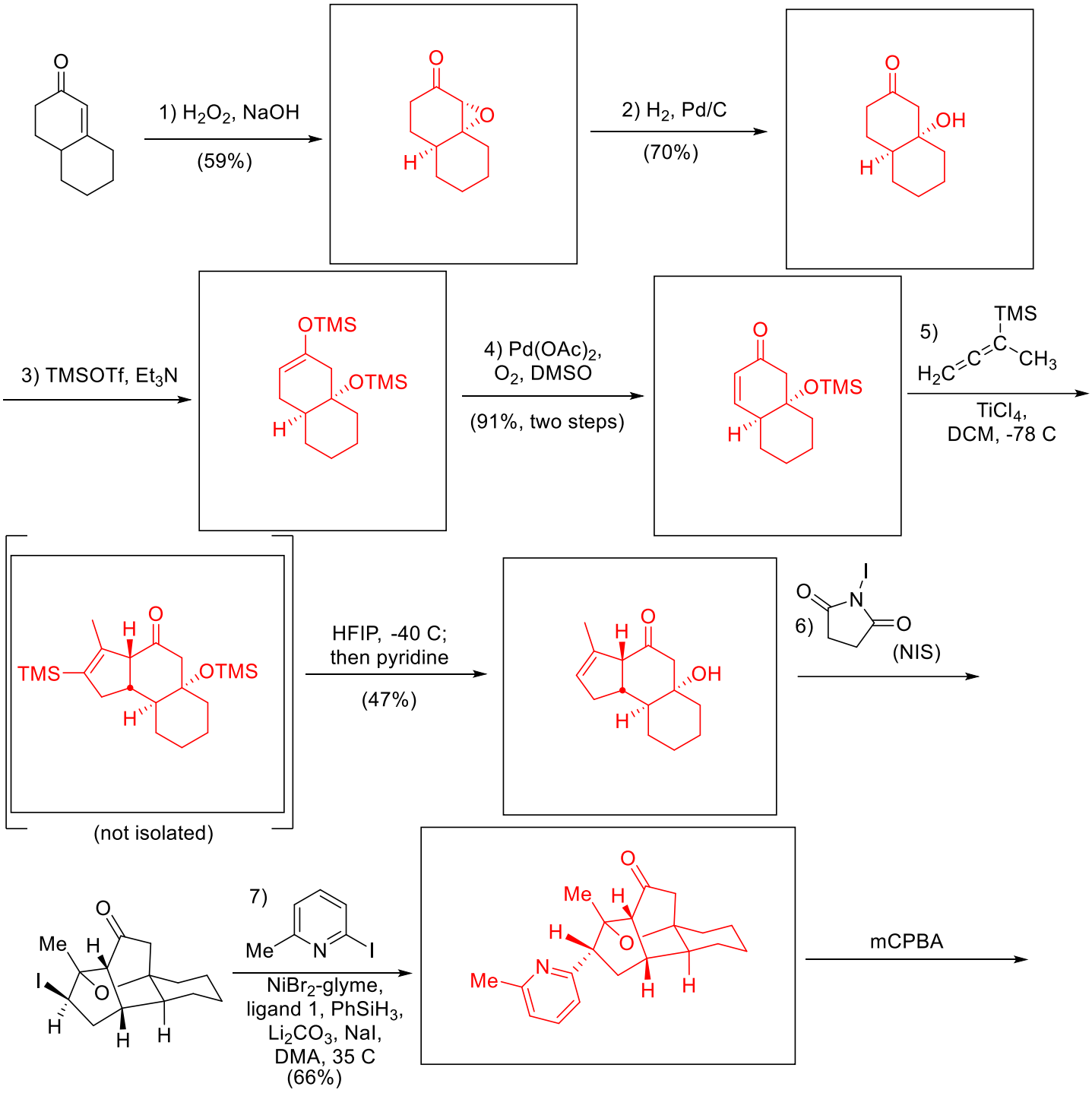
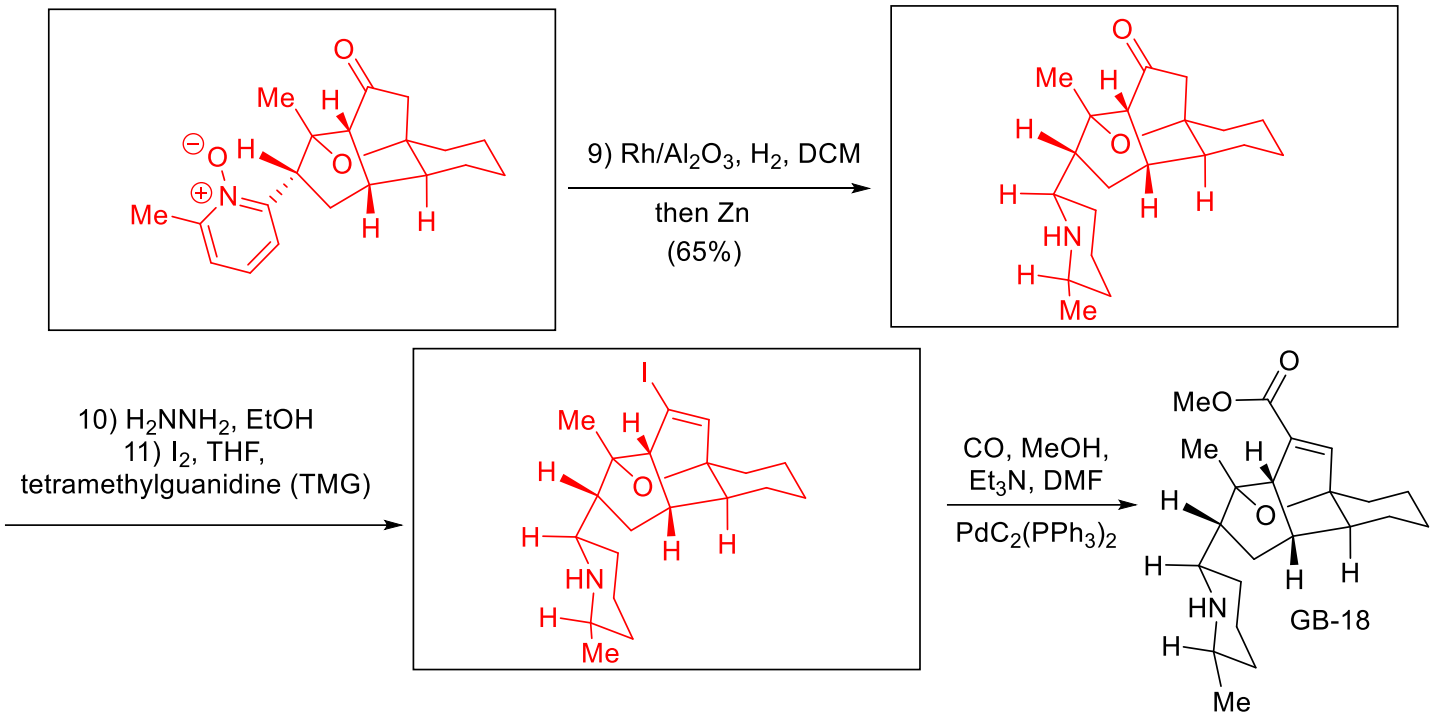


Figure 4a shows a screen done to analyze the receptors that GB-18 can bind. The different neurological receptors analyzed in the extensive screen are listed along the x-axis. The y-axis measures the impact GB-18 has on each of the different receptors relative to the binding of the natural ligand. A radioactive natural ligand was used and permitted a quantitative assessment of GB-18 binding. The amount of radioactive ligand displaced or inhibited from binding by GB-18 determines the affinity and potency of GB-18 for each of the different receptors. GB-18 showed the highest affinity for the mu and kappa opioid receptors and some affinity for the delta opioid receptor.

c) Provide <u>five products or intermediates</u> on the pathway to the synthesis of GB-18. You only need to provide five correct products or intermediates (2 pts. each) for full credit, and it can be any five. Incorrect stereochemistry will be a minor deduction. (10 pts.)


1) H_2O_2, NaOH
(59%)
2) H_2, Pd/C
(70%)
3) TMSOTf, Et_3N
4) $Pd(OAc)_2$, O_2, DMSO
(91%, two steps)
5)
$TiCl_4$, DCM, -78 C
HFIP, -40 C; then pyridine
(47%)
6)
(NIS)
(not isolated)
7)
$NiBr_2$-glyme, ligand 1, $PhSiH_3$, Li_2CO_3, NaI, DMA, 35 C
(66%)
mCPBA



7. Dr. Denise Grünenfelder, Bristol Myers Squibb, April 8: "The Frontier of T Cell Checkpoint Therapies: Discovery of Dual Inhibitors of Diacylglycerol Kinases α and ζ" *Lecture reference*: Discovery of Potent, Dual-Inhibitors of Diacylglycerol Kinases Alpha and Zeta Guided by Phenotypic Optimization, *ACS Med. Chem. Lett.* **2023**, *14*, 7, 929–935.

a) What are T-cell checkpoint therapies like Opdivo and what role does diacylglycerol kinase (DGK) play in checkpoint therapies and cancer treatment? (3 pts.)

Checkpoint therapies attempt to stop cancer's inhibition of an immune system response. DGK phosphorylates diacylglycerol (DAG), which suppresses an immune response to cancer by preventing the release of various immunological factors. Preventing this phosphorylation event allows for a more robust immune response to cancer cells.

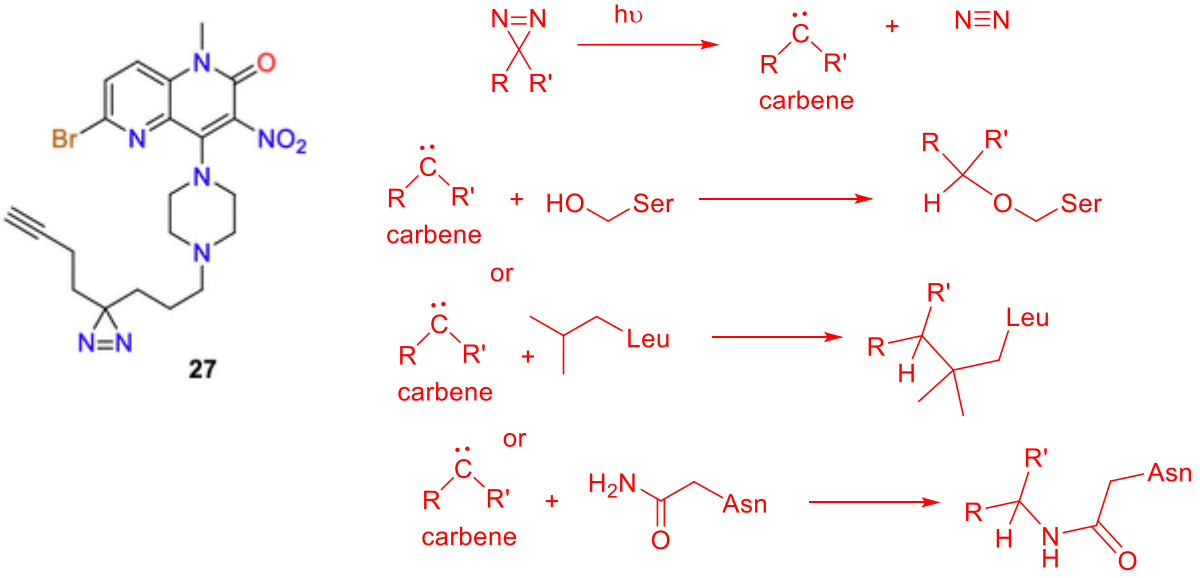
b) Several drug development efforts discussed this semester, including that presented by Dr. Grünenfelder and her co-workers with diacylglycerol kinase (DGK) inhibitors, used phenotypic screening in their drug development programs. Briefly explain the nature of phenotypic screening and how it differs from target-based screening. (3 pts.)

Phenotypic screening analyzes physiological responses to determine the impact of a particular molecule or potential drug on a cell, organism or animal. Target-based screening has a known protein target, usually an enzyme or receptor, which is often isolated, and an associated target protein assay that can specifically monitor the catalytic or physiological activity of that isolated protein after exposure to the potential drug.

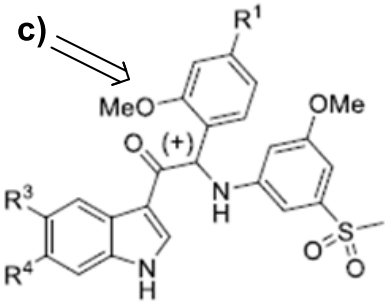
c) After an active and potent lead compound is discovered, what experiments are typically done to find the target of the lead compound? (3 pts.)

Usually, after identifying a lead compound, the researchers expose cells to relatively low levels of the lead compound over extended periods of time and then analyze the cell's DNA for mutations. As the cell tries to evolve and thrive in the presence of the lead compound, the cell mutations made to overcome the lead compound's inhibitory or cytotoxic effects are usually found with the protein that the lead compound targets or inhibits.

d) Dr. Grunenfelder described the use of diazirine compound (compound **27** below) by her Bristol-Myers Squibb colleagues to find the site of binding for their DG kinase inhibitor.
(i) Show the reaction of a generic alkyl diazirine with light. Draw a Lewis dot structure of the product to properly show its structure. Include any side products from the reaction. (3 pts.)
(ii) Show a reaction between your product in part **i**, i.e. the reactive product of diazirine photochemical decomposition, and an amino acid side chain. You may use any amino acid side chain. (3 pts.)



8. "Discovery of JNJ-1802, a First-in-Class Pan-Serotype Dengue Virus NS4B Inhibitor" *J. Med. Chem.* 2024, 67, 5, 4063–4082, Tim H.M. Jonckers et al. *(Rio and Simone, April 10)*
In the article on a pan-serotype dengue virus NS4B inhibitor, the Johnson & Johnson researchers showed the following structure and table.



compd	R^1	R^3	R^4	DENV-1 [a] EC_{50} (μM) [b]	DENV-2 [c] EC_{50} (μM) [b]	DENV-3 [d] EC_{50} (μM) [b]	DENV-4 [e] EC_{50} (μM) [b]	hLM CL_{int} (μL/min/mg protein)	hHep CL_{int} (μL/min/10^6 cells)
8a	F	H	H	0.012	0.0020	0.12	0.23	9	7.0
10a	F	H	F	0.0081	0.0013	0.050	0.17	<8	<1.9
11a	Cl	H	F	0.0025	0.00028	0.023	0.093	<8/10 [f]	<1.2
12a	F	Me	OMe	0.0045	0.00061	0.055	0.11	<8	7.6
13a	Cl	Me	OMe	0.00097	0.00020	0.015	0.053	<8	<1.9

a) What is DENV-1, DENV-2, DENV-3 and DENV-4, and why were all four being tested? What was the goal of the drug development process relative to these four items in the table? (3 pts.)
The DENV-1 through 4 are the four different serotypes of the dengue virus and the researchers wanted to be sure to have a "pan" inhibitor that would kill all four serotypes of the dengue virus. It is critical to block all four

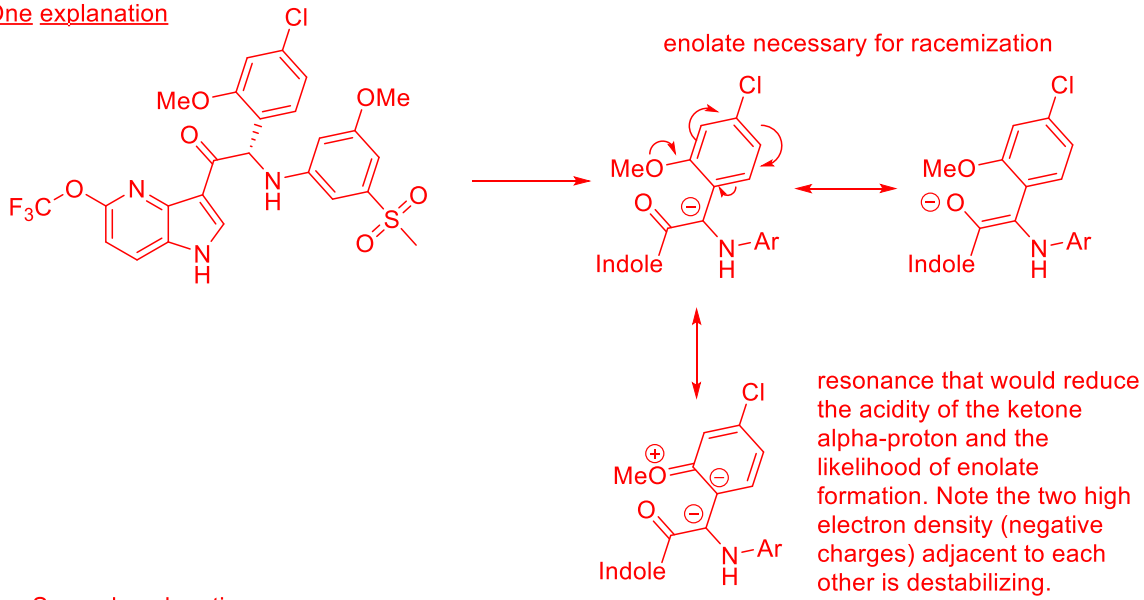
virus serotypes as a second infection, by another serotype, can be more devastating for a person's health than the first.

b) What did the (+) next to the ketone indicate? (3 pt.)

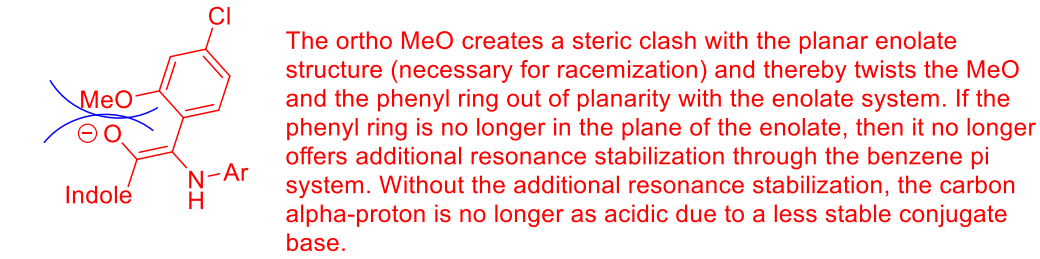
The (+) indicated the adjacent carbon was a chiral center and was unstable and susceptible to racemization from one enantiomer to the other enantiomer.

c) What role did the ortho-methoxy group (see **c)** →in the structure picture above) play in relation to the (+) issue from part **b**? Your explanation should show resonance forms and/or reaction intermediates to illustrate your point. (5 pts.)

One explanation



Second explanation



d) What did the hLM Cl_{int} and hHep Cl_{int} tell the researchers? Your answer should specifically address the differences between the five compounds shown in the table, the meaning of the Cl property and what value this information had for the overall drug development goals. (4 pts.)

The hLM Cl_{int} and hHep Cl_{int} are the human liver microsome clearance and human hepatocytes clearance rate of the different molecules, **8a-13a**. The values show that the clearance rate or metabolism of **10a**, **11a** and **13a** were low and that for **8a** and **12a** was higher. Therefore **10a**, **11a** and **13a** would likely be stable molecules if used as drugs and not be degraded or removed from the system too quickly. Metabolic stability is an important goal in developing a new drug to be sure the drug will be able to exert its therapeutic effect before it is degraded by the body.

9. "Discovery of Taniborbactam (VNRX-5133): A Broad-Spectrum Serine- and Metallo-β-lactamase Inhibitor for Carbapenem-Resistant Bacterial Infections" *J. Med. Chem.* 2020, 63, 6, 2789–2801. B. Liu, C. J. Burns et al. (Venatorx Pharmaceuticals in Malvern, PA) *(Nuha and Kyra, April 15)*

a) What are four common methods that bacteria have developed to evade beta-lactam antibacterial drugs? Briefly explain each. (4 pts.)

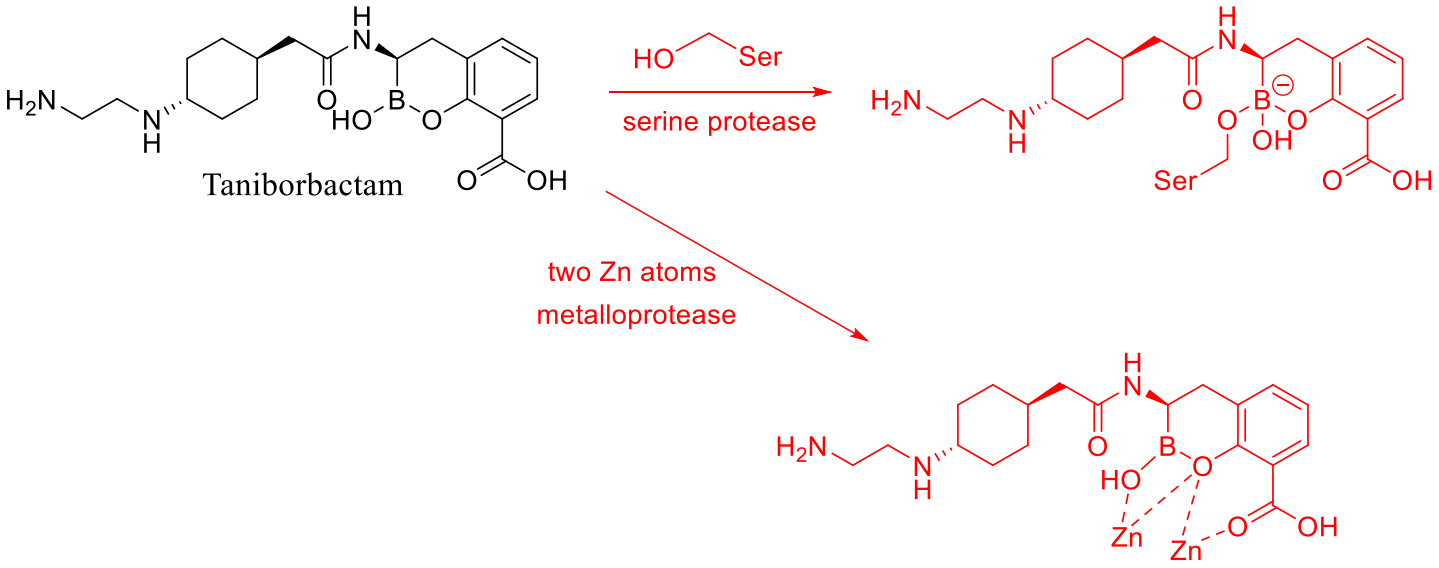
Bacteria increase the number of efflux pumps on their membranes to pump the beta-lactam drugs out of the bacterial cell

Penicillin-binding proteins (PBP's), which are the site of therapeutic action of beta-lactam drugs, are mutated or modified to reduce the affinity of beta-lactams for the PBP's.

Bacteria modify porin channels to reduce the penetration of beta-lactam drugs into the cell.

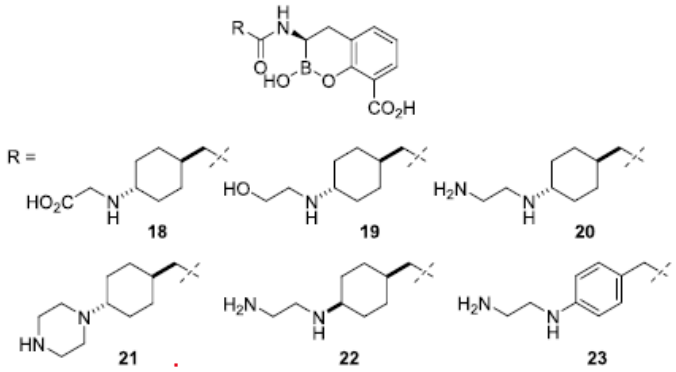
Bacteria increase the number of beta-lactamase enzymes which cleave the critical and reactive beta-lactam ring present in beta-lactam drugs.

b) As discussed in the article and presentation, Taniborbactam inhibits both serine- and metallo-beta-lactamase enzymes. Show the structure of Taniborbactam as it inhibits both types of lactamase enzymes. Your picture should focus on the key interaction with the enzyme catalytic residues or atoms. You need not show other intermolecular interactions. (5 pts.)



c) Table 2 from the article is reproduced below. Answer the two questions below about the data shown in Table 2.

Table 2. Biochemical and Microbiological Activity of BLIs 18−23



			biochemical assay, IC_{50} (μM)[b]				microbiological assay, MIC (μg/mL)[a]			
							K. pneumoniae			*P. aeruginosa*
entry	clogP	tPSA	KPC-2 (class A)	AmpC (class C)	OXA-48 (class D)	VIM-2 (class B)	KPC-2	OXA-48	VIM-4	VIM-2
18	−1.85	145	0.019	0.006	3.14	2.4	4	8	32	16
19	−1.99	128	0.017	0.01	7.55	1.4	2	2	0.5	4
20	−2.39	134	0.03	0.032	0.42	0.02	0.5	2	0.5	0.06
21	−1.32	111	0.02	0.003	1.52	1.5	4	16	32	16
22	−2.39	134	0.03	0.035	0.67	0.13	8	8	4	0.5
23	−1.81	134	0.022	0.005	0.288	0.491	1	1	8	0.5

[a]Reported as the BLI concentration able to restore meropenem (fixed at 4 μg/mL) antibacterial activity. [b]Results are expressed as the average from duplicates unless otherwise indicated.

(i) What is so striking about the clogP values listed for the compounds relative to other drugs that we've discussed this semester and to the general guidelines around typical clogP values for most successful drugs? What do the values say about the nature of the compounds in the table? (3 pts.)

The clogP values are quite low, much lower than the 2-5 values typically seen for most drugs. Therefore, these compounds are much more hydrophilic than the typical drug.

(ii) In the biochemical assay for inhibition of OXA-48 (column 6), compounds **18**, **19** and **21**, have a much higher IC_{50} than **20**, **22** and **23**. Analyze the structures of **18**, **19** and **21** and suggest structural characteristics that might lead to weaker activity. You should speculate about the different type of interactions with the OXA-48 enzyme that might be lost and lead to the weaker enzyme inhibition. You can assume that enzyme and drug compounds are in an aqueous solution at physiological pH ~7.4. (3 pts.)

Compounds **18** and **19** lack an amino group and instead have a carboxylic acid and an alcohol. The amino group of compounds **20**, **22** and **23** is likely to exist as an ammonium ion at physiological pH and may interact with a negatively charged group. Neither the carboxylic acid of **18** nor the alcohol of **19** would be able to satisfy that ionic interaction. Compound **21** would be able to provide an ammonium ion, but the ring structure likely constrains the amino group, and the restricted rotation may make it difficult for an optimum interaction to be formed between the ammonium ion and the complementary amino acid group in the OXA-48 enzyme.

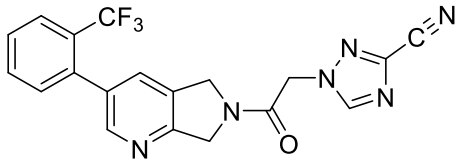
10. "Cyanotriazoles are selective topoisomerase II poisons that rapidly cure trypanosome infections". Science 29 Jun 2023, Vol 380, Issue 6652, pp. 1349-1356 S. P. S. Rao et al. *(Paige and Taylor, April 17)*

a) The following kinetic and thermodynamic data (**B**, Figure 5B from the article) was generated and it quantitatively evaluates the interaction of the drug, CT1, with the target(s). Briefly explain the inhibition event between CT1 and its target(s). What is it binding? How does this kill the trypanosome parasite? What do the different kinetic and thermodynamic parameters say about the interaction of the CT1 and its target(s)? (5 pts.)

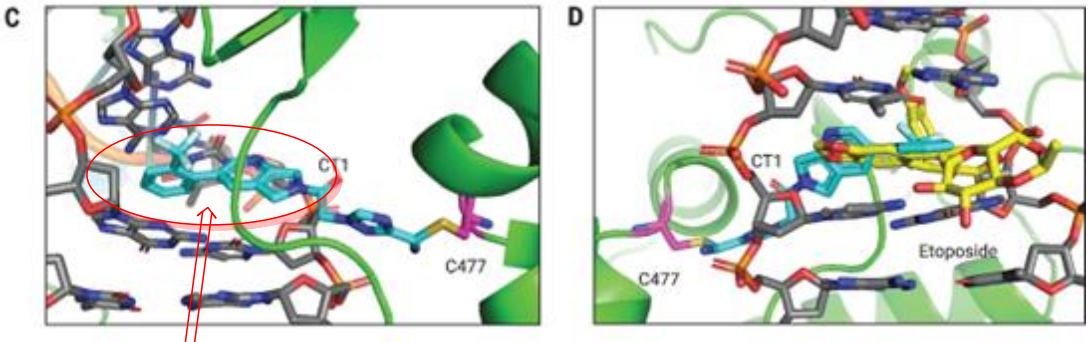
	CT1	DMSO
k_a(1/Ms)	5.91e+05	6.12e+04
Rmax (RU)	2263.8	2200.0
k_d (1/s)	5.95e-04	8.15e-02
K_D (M)	1.01e-09	1.33e-06

CT1 binds together the double-stranded (ds) nuclear DNA of the parasite and the topoisomerase II enzyme, which normally serves to relax supercoiled ds DNA. This binding prevents ds DNA from dissociating and completing the supercoiling relaxation process or conducting any more catalytic relaxation events. The dissociation equilibrium constant, K_D, shows that the ds DNA and topo II are bound much more tightly with CT1 present than with just DMSO solvent ($1x10^{-9}$ vs $1.33x10^{-6}$ M). The k_d dissociation rate constant shows that the rate of dissociation of the ds DNA and the topo II is much slower ($6x10^{-4}$ vs $8x10^{-2}$ s^{-1}) in the presence of CT1 versus without CT1 (DMSO).

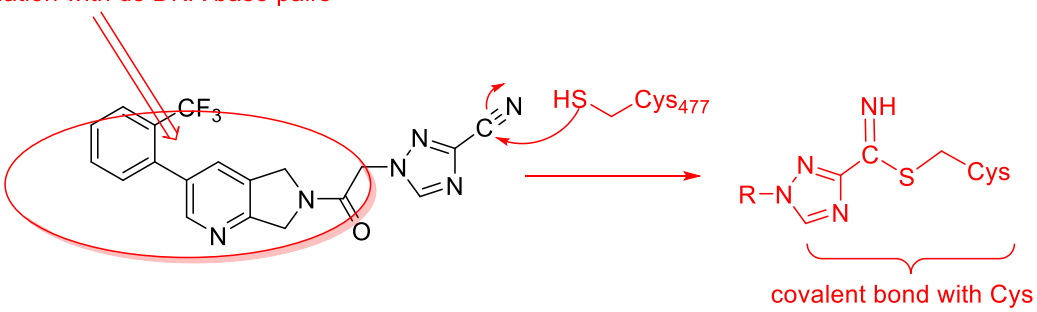
b) What is/are the primary binding event(s) that lock CT1 into its target(s)? Show with structural drawings and/or brief explanations the key binding activities that are critical to CT1's mechanism of action. (6 pts.)



CT1



intercalation with ds DNA base pairs

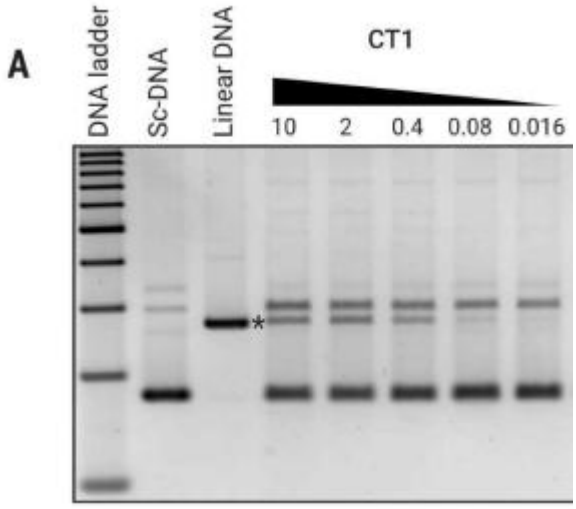


covalent bond with Cys

As shown above, there is one important interaction of the CT1 with the ds DNA and one with the topo II. CT1's aromatic benzene and pyridine regions (circled) intercalate between the DNA base pairs in the ds DNA. The nitrile group on the right side of the CT1 molecule reacts with a cysteine 477 thiol side chain to trap the thiol in a covalent bond with the nitrile group as shown.

c) A portion of Figure 5A from the article is reproduced below. What does the data say about CT1's biological activity? Your answer should address what the numbers 10, 2, 0.4, 0.08 and 0.016 represent. (4 pts.)

The data show the amount of DNA damage as measured by the prevalence of linear DNA in the presence of CT1. With increasing concentrations of CT1 (0.016, 0.08, 0.4, 2, and 10 μM), there is more and more linear DNA created, a sign that the CT1 is preventing or aborting the normal activity of topo-2 enzyme. Since CT1 traps the ds DNA in a complex with topo-2 and prevents the re-ligation of the DNA, the presence of CT1 is generating linear DNA.



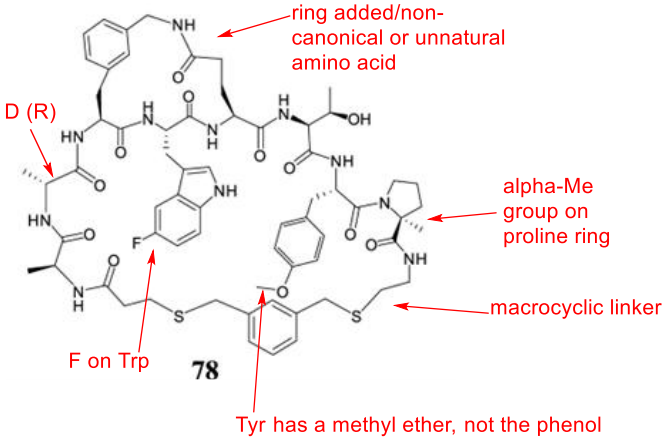
11. Dr. Dipa Kalyani, Merck, April 22: "Cyclic peptides and drug development for coronary artery disease" *Lecture reference*: Series of Novel and Highly Potent Cyclic Peptide PCSK9 Inhibitors Derived from an mRNA Display Screen and Optimized via Structure-Based Design. *J. Med. Chem.* **2020**, *63*, 22, 13796–13824

a) Peptide drugs are often considered a problem for oral delivery. Why is that? (3 pts.)

Peptide or amide bonds can be readily hydrolyzed or cleaved by protease enzymes found in the digestion system. That means most peptide drugs are metabolized in the gut before reaching their site of action or target protein.

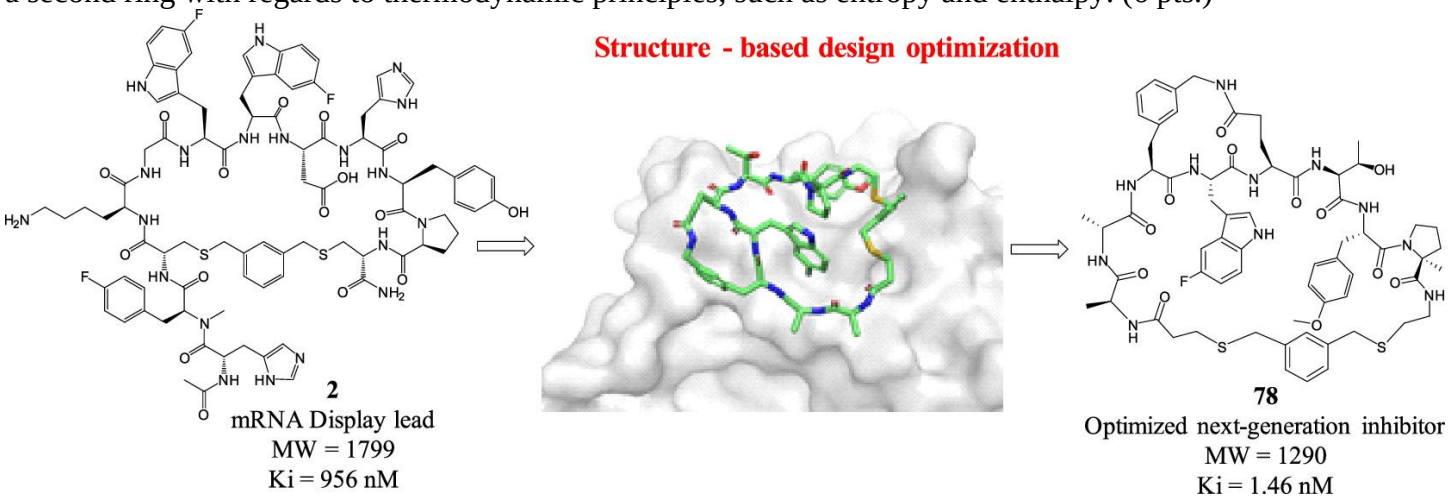
b) Compound **78** (below) is the macrocyclic peptide, which was developed in the article and discussed by Dr. Kalyani, and it did show generally good stability for oral delivery. Cite four structural modifications that likely contributed to compound **78's** greater stability. (6 pts.)

Possible answers:
1) Compound 78 has a D-alanine amino acid. D-amino acids are less susceptible to protease degradation since they are unnatural or non-canonical.
2) There is a F substituted on the Trp indole ring making it unnatural.
3) There is ring formed at the top that ties a Gln and a Phe together making for an unnatural side chain.
4) There is an alpha-Me group added to the Pro alpha-carbon.
5) The Tyr has a methyl ether where it is normally a phenol, so it is also unnatural.



6) The structure is a macrocycle which is itself less susceptible to degradation since it is not the usual conformation of most peptides.

c) The image below from the literature article abstract discussed by Dr. Kalyani shows the evolution of the lead compound **2** to the optimized PCSK9 inhibitor **78**. The transformation included adding a second ring along the top of the structure, as shown in compound **78**. Briefly describe the potential costs and benefits of incorporating a second ring with regards to thermodynamic principles, such as entropy and enthalpy. (6 pts.)



If the conformation is pre-organized in the active conformation of the drug, then there is a lower entropy penalty, i.e. less negative ΔS, for the drug to enter the target and bind. There would be no need for additional structural organization since the drug is already in the proper conformation.

With regards to enthalpy, there would be benefits if the hydrocarbon piece installed had additional intermolecular forces or interactions, e.g. van der Waals interactions, with a portion of the binding site location. Greater occupation of empty space can also benefit the binding energy enthalpy.

12. "Diversity-oriented synthesis yields novel multistage antimalarial inhibitors". Nature 2016, Vol. 538, pp. 344-349. S. L. Schreiber et al. *(Chelsea and Bernie, April 24)*
a) What is diversity-oriented synthesis and how was it used in the research reported to achieve a new antimalarial drug? (3 pts.)

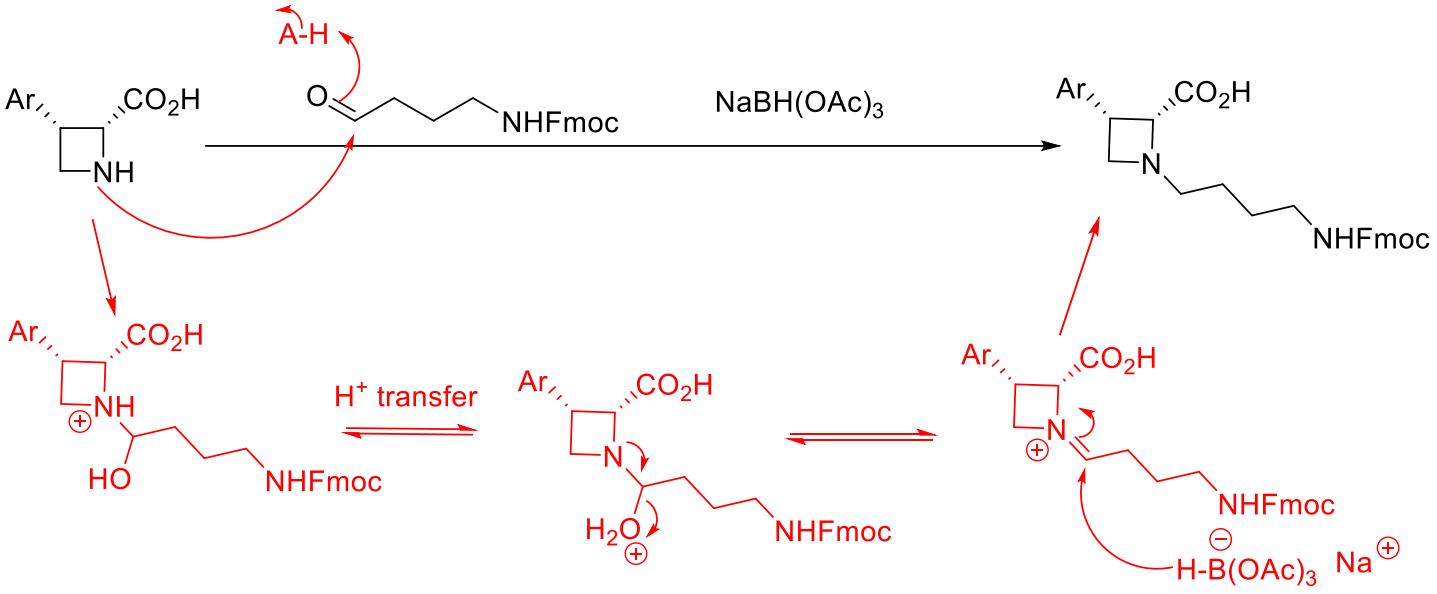
Diversity oriented synthesis is a strategy to use synthetic chemistry tools to create a large variety of structurally different and complex (natural product-like) molecules quickly with the goal of analyzing a large amount of chemical space and discovering new bioactive or therapeutically active molecules, often in phenotypic screens.

b) Describe the anti-malarial mechanism of action of BRD7929 by answering the following four questions. One sentence should suffice for each. (5 pts.)
(i) What was the parasite target of the bicyclic azetidine drug BRD7929 and what does that target protein normally do?
The target of BRD7929 was phenylalanine tRNA synthetase, which is responsible for the synthesis of phenylalanine-tRNA that is critical for the construction of proteins with phenylalanine amino acids.

(ii) Where does the BRD7929 bind on the target?
BRD7929 binds in both the ATP pocket and the phenylalanine amino acid binding pocket of tRNA synthetase.

(iii) How was this report of BRD7929 binding to the parasite target a seminal discovery?
The parasite's phenylalanine tRNA synthetase was never previously recognized as a potential target, so BRD7929 was able to identify a new malaria parasite therapeutic target.

c) Show the mechanism of the following reaction, a reductive amination. (7 pts.)

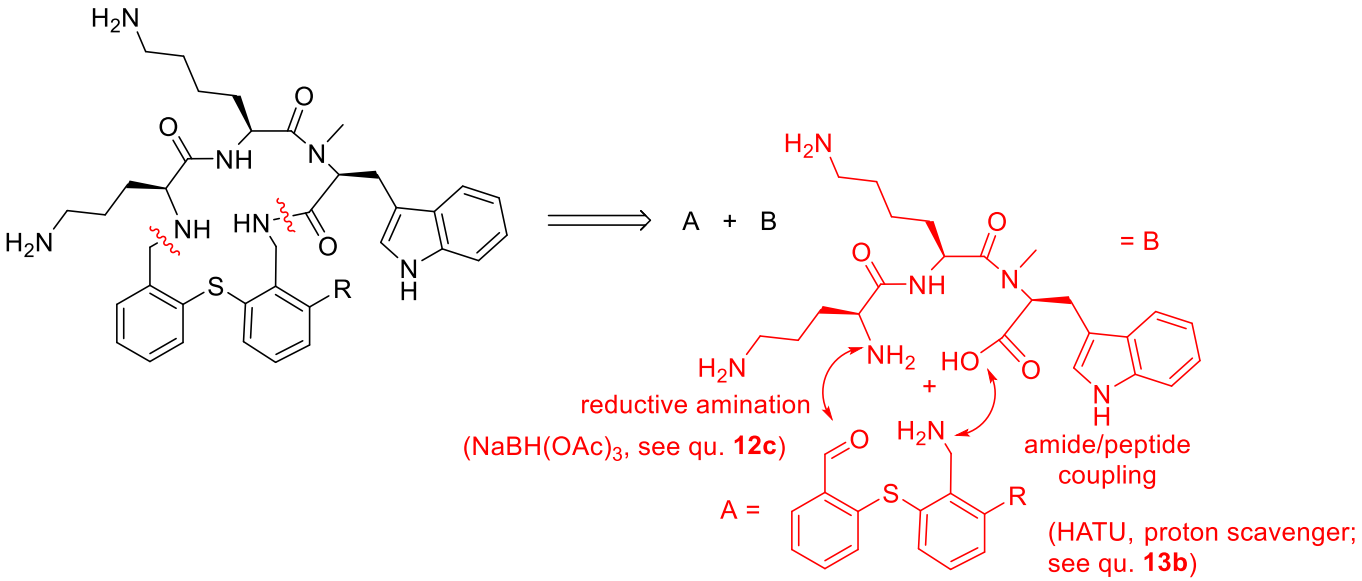


13. "A novel antibiotic class targeting the lipopolysaccharide transporter". Nature, *volume 625*, pages 566–571, (2024). C. Zampaloni, M. A. Lobritz, K. A. Bradley et al. (Hoffman-La Roche Pharmaceutical Company) *(Sonja and Eliana, April 29)*

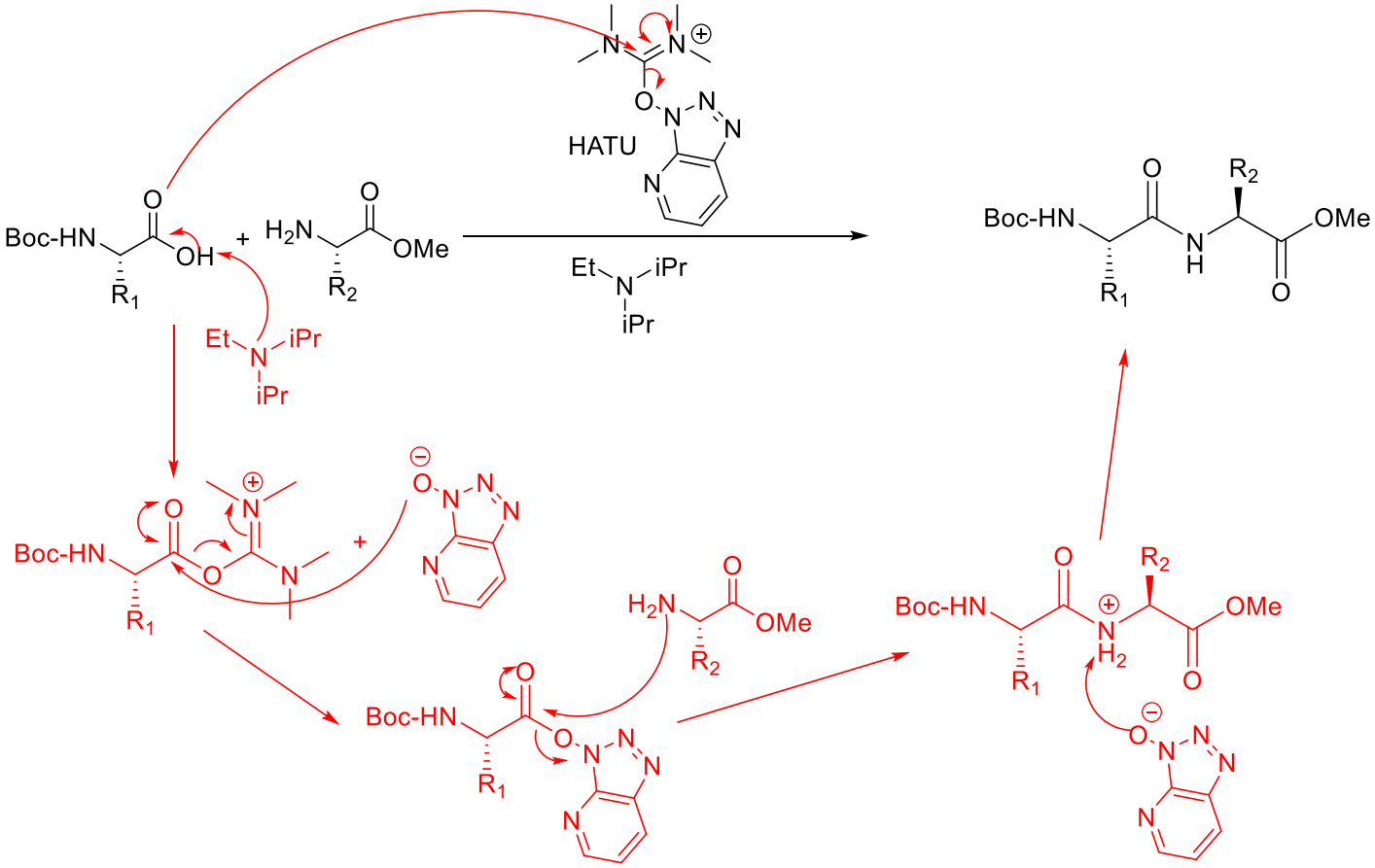
a) Over the course of the semester, we saw several different bacteria targets for current antibacterials. Briefly explain what they were and how the macrocyclic peptide drug Zosurabalpin (ZAB) was similar or was different. (5 pts.)
We saw two traditional bacteria targets of current antibacterials: cell wall biosynthesis and ribosome protein synthesis. Beta-lactam antibacterials inhibit cell wall biosynthesis by blocking the enzyme D-Ala-D-Ala transpeptidase. The bacterial ribosome was the target of the iboxamycin (IBX) or clindamycin drugs discussed earlier in the course. The ribosome is responsible for bacteria protein synthesis, therefore ribosome inhibition prevents bacterial protein synthesis.

ZAB targets the lipopolysaccharide (LPS) transporter system and binds to one of the proteins (LptF) preventing the movement of critical lipopolysaccharides from the interior of the bacterial cell to the exterior cell wall, which is critical for bacterial survival. Therefore, ZAB is like the beta-lactam antibiotics in targeting cell wall biosynthesis, but it inhibits a transporter protein, not the enzyme transpeptidase.

b) Show a convergent synthesis of the macrocyclic peptide ZAB. You can use the one by the Hoffman-La Roche researchers and described by Sonja and Eliana or you may choose another version. Show two parts of the molecule (A + B) that could be combined to create the final ZAB structure. Suggest two reactions to create the final two bonds for your convergent synthesis to combine substructures A and B. Show key reagents that you could use to accomplish those two reactions. You are encouraged to abbreviate your structures A and B. (4 pts.)

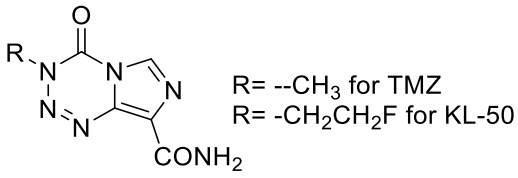


b) Provide a mechanism for the following amid-bond-forming reaction. (6 pts.)



14. "Mechanism-based design of agents that selectively target drug-resistant glioma". Science 2022, Vol. 377, pp. 502-511. R. Bindra, S. Herzon et al. *(Gabby, Joseph and Spencer, May 1)*

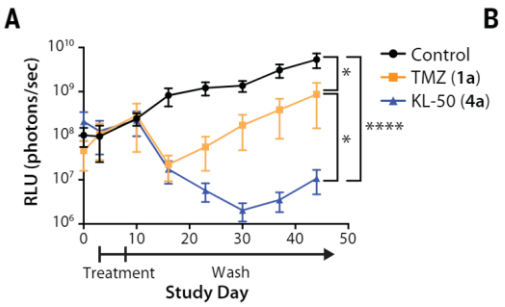
a) What is the reaction of temozolomide (TMZ) with DNA, which serves as a treatment for glioblastoma? Briefly explain how this reaction helps to stop the glioblastoma. You do not need to show any reaction, mechanism or products. (3 pts.)



Temozolomide causes the methylation of DNA at a variety of sites. The methylation initially leads to DNA repair mechanisms fixing problems and attacking glioblastomas.

b) Figure 6A from the article is reproduced below. What does the figure tell us about TMZ and KL-50? Be sure to address why the study was done specifically on an MGMT-/MMR- intracranial tumor. Also please comment on the unique mechanism of KL-50. Note: MGMT- is O^6-methylguanine methyl tranasferase deficient tumor and MMR- is a mismatch repair deficient tumor. (5 pts.)

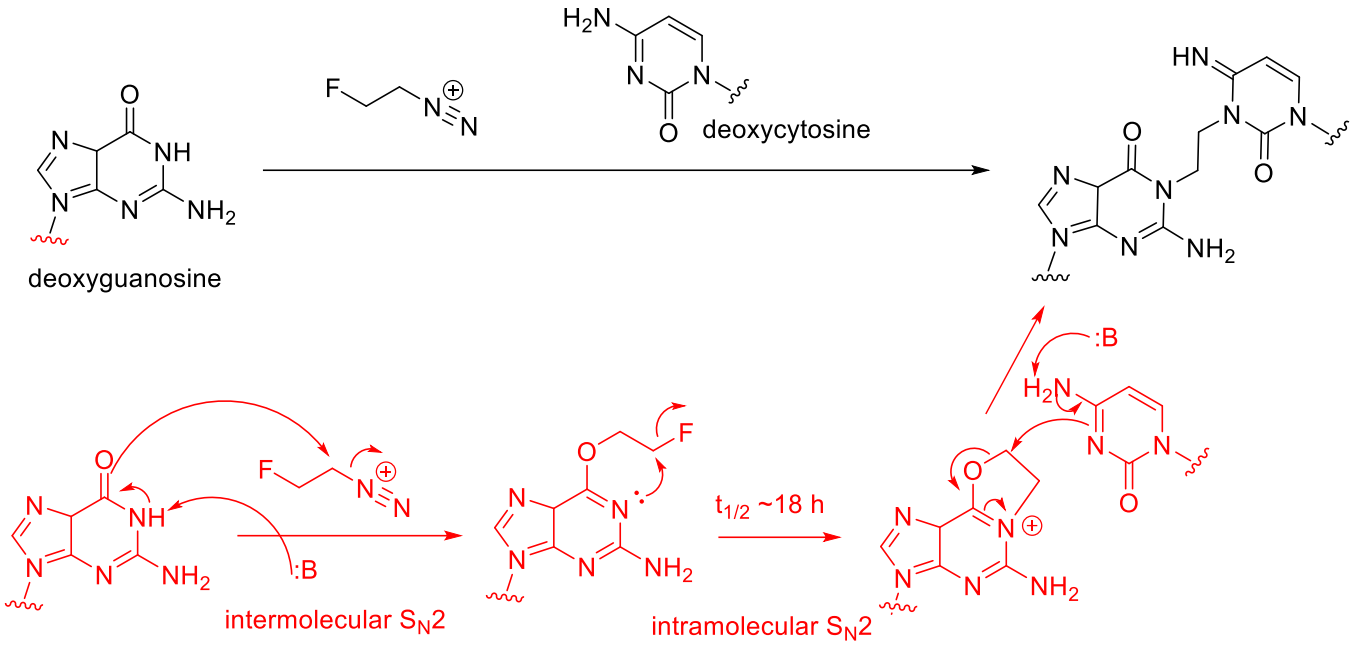
Figure 6A



The legend reads, "Mean tumor size as measured with bioluminescent imaging as relative light units (RLU; photons/s) with SEM of xenograft LN229 MGMT−/MMR− intracranial tumors treated with 5 consecutive days of oral administration of 10% cyclodextrin control (n = 10 mice), TMZ (1a) (n = 11 mice, 25 mg/kg), or KL-50 (4a) (n = 11 mice, 25 mg/kg)."

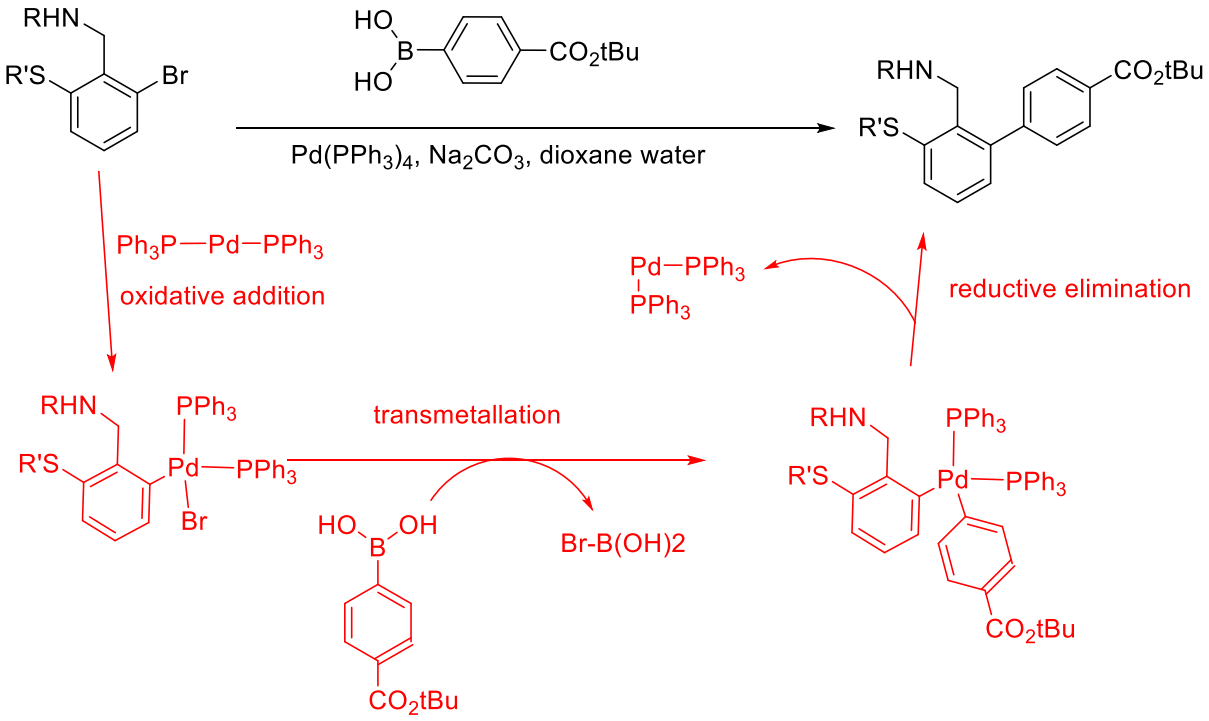
The figure shows a study of the effects of the drugs TMZ and KL-50 on a tumor. The control is the experiment without any drug and allows a comparison of the effects of TMZ and KL-50. From the graph, TMZ causes an initial decrease in the tumor size during treatment, but the tumor growth returns approximately 10 days after treatment. KL-50 demonstrates much stronger tumor suppression over a longer period of time, but the tumor does start to regrow roughly 20 days after treatment. The study was conducted with MGMT-/MMR- tumors because those are difficult to treat and the purpose of developing the new drug, KL-50. KL-50 has a new mechanism of action that not only O-alkylates guanosine, but also, over time, forms interstrand cross-links between guanosine and cytosine as shown in the mechanism of part **c** (below). After interstrand cross-links are formed, the DNA is damaged enough (i.e. lesions) to prevent the tumor DNA from replicating and the tumor growth is suppressed.

c) Show the curved arrow electron flow mechanism for the reaction of KL-50's 2-fluoroethyl diazonium ion, liberated after hydrolysis and decarboxylation of KL-50, in forming interstrand cross-links (ICL's) between a guanosine and a cytosine as described in the article. You may assume acids and bases are available to facilitate the process. (7 pts.)

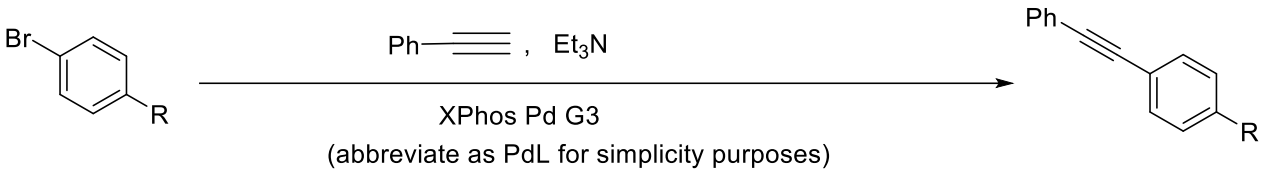


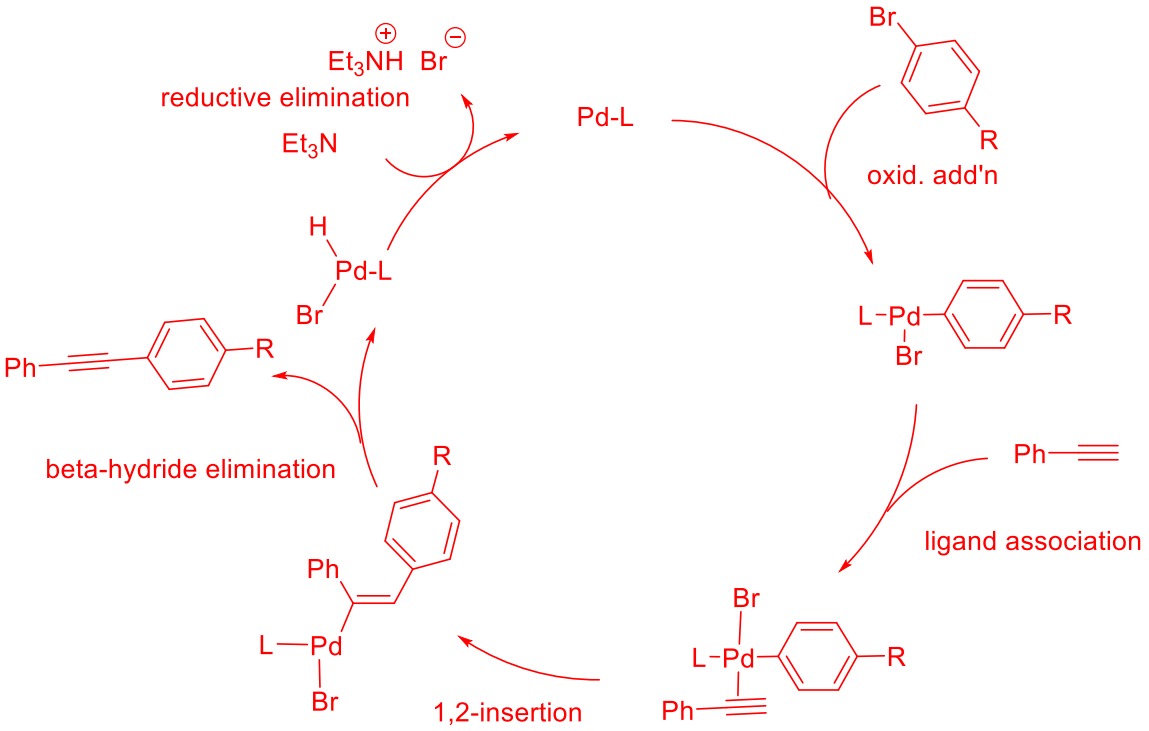
15. Organometallic mechanisms. Choose **one** of the two reactions below and show the mechanism of the catalytic cycle. Identify all the steps by the type of organometallic mechanistic process. You are encouraged to abbreviate structures but be sure to show the key components. Provide the name of the reaction that you choose for demonstrating the catalytic cycle. (15 pts.)

a) The Suzuki-Miyaura reaction from Sonja and Eliana's presentation.



b) Show the mechanism of the Heck alkynylation reaction used in the synthesis of BRD7929 and presented by Bernie and Chelsea. Be sure to label each step of the catalytic cycle with the appropriate mechanistic name.


Br
R
Ph
, Et3N
XPhos Pd G3
(abbreviate as PdL for simplicity purposes)
Ph
R


Et3NH Br
reductive elimination
Et3N
Pd-L
Br
R
oxid. add'n
H
Pd-L
Br
L-Pd
R
Br
Ph
R
beta-hydride elimination
Ph
ligand association
R
Ph
L—Pd
Br
Br
L-Pd
R
Ph
1,2-insertion