Chemical Biology Problem set #1

1. Please do your best to know the structures of all 20 naturally occurring amino acids.

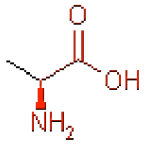
2. HBTU (also known as 2-1H-benzotriazole-1-yl)-1,1,3,3,-tetramethyluroniumhexafluorophosphate) is commonly used as a coupling reagent in solid phase peptide synthesis.

    a. Draw a mechanism showing how HBTU works.
    b. It has been observed that a mixture of HOBt (also known as N-hydroxybenzotriazole) and HBTU leads to coupling reactions that proceed even faster than those containing HBTU. Why?

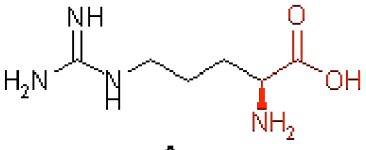
3. A Boc protecting group can be installed on the N-terminus of an amino acid using the reagent known as Boc-On (also known as 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile) whose structure we learned in class. Draw a mechanism showing how Boc-On works.

4. The Rink resin is used frequently to synthesize peptides whose C-termini are modified as primary amides (instead of free carboxylic acids). Draw structures and arrows illustrating how treatment of a peptide synthesized on a Rink resin with strong acid (95% trifluoroacetic acid) cleaves the peptide from the resin in a way that leaves an amide at the C-terminus.

(L)-amino acids Know these structures!!



Ala



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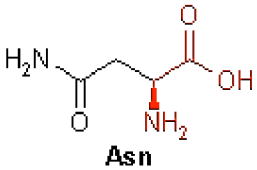
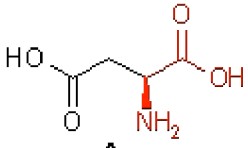
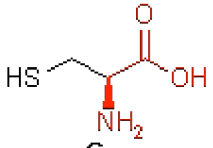


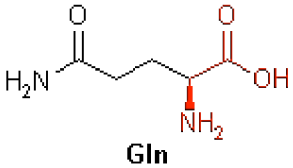
Asn



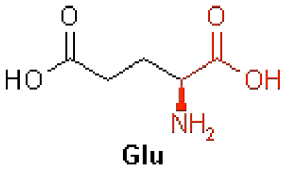
Asp



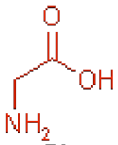
Cys



Gln



Glu



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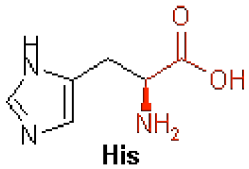
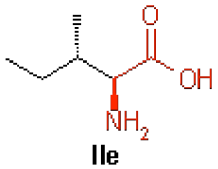
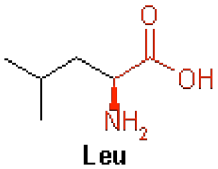


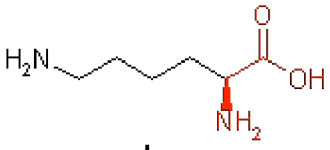
His



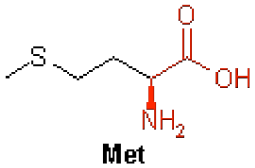
Ile



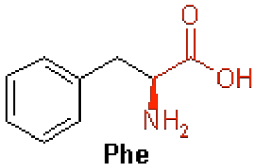
Leu



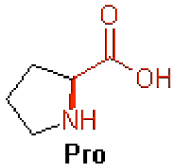
Lys



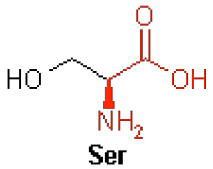
Met



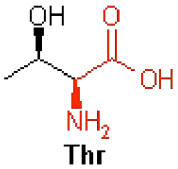
Phe



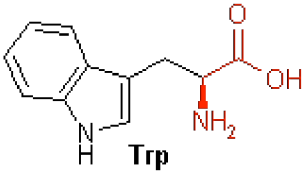
Pro



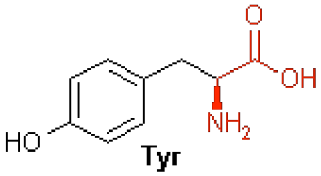
Ser



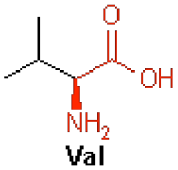
Thr



Trp



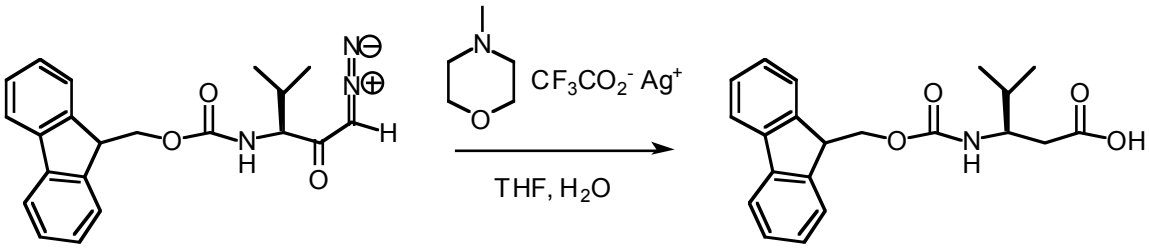
Tyr



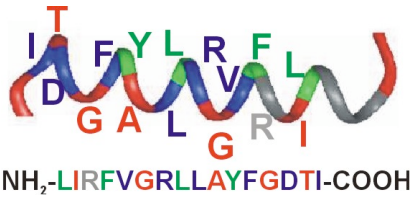
Val

Chemical Biology Problem set #2

1. Consider the Arndt-Eistert and Anna Mapp methods for synthesizing β-amino acids.
   a. Write 3-4 sentences that describe the advantages and disadvantages of each method.
   b. Draw the mechanism for the Wolff Rearrangement of the diazoketone below to form the corresponding β-amino acid.



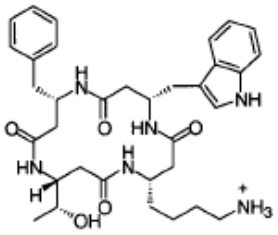
2. Consider the sequence of the α-helix shown at right. The residues in blue contribute to the biological function of this peptide, whereas those in red and green do not.



   a. Design a foldamer composed of β-amino acids that could recapitulate the biologic function of the α-helix. Your design should reflect the fact that only a fraction of the residues in this peptide contribute to function. The molecule you design can be 14-helical or 10/12 helical, but should obey the known parameters that stabilize the respective secondary fold.
   b. Label the residues on your foldamer as helix stabilizing, binding, or unimportant.

3. Explain why β-peptides are resistant to cellular proteases.

4. Consider the β-peptide shown below.



   a. Is this molecule a foldamer? Justify your answer in 2-3 sentences with your own interpretation of what it takes to be called a foldamer.
   b. What is the logical next "step" for the foldamer field as you see it? What is left to prove? What are the new challenges? (2-3 sentences)

Chemical Biology
Problem set #3

1. Describe the chemical steps necessary to convert a natural tRNA into a tRNA charged with a non-natural amino acid.

2. The indicator reagent X-Gal is used commonly to distinguish bacteria that have been successfully transformed with a given plasmid (one that contains the *lacZ'* gene) from those that have not.

    a. What is the chemical reaction that X-Gal undergoes in the presence of b-galactosidase? What color change occurs?
    b. The *lacZ'* gene encodes only an amino-terminal fragment of b-galactosidase. Where does the remainder of the protein come from? [Look up alpha-complementation.]
    c. Can you imagine a scenario in which the color change you describe in (a) occurs but the bacteria does not, in fact, contain the requisite plasmid?
    d. Can you imagine a scenario in which the color change you describe in (a) does not occur but the bacteria does, in fact, contain the requisite plasmid?

3. PCR is a wonderful tool for making large quantities of desired DNA. In the equation:

$$\#\text{ of copies (i.e. the "amplification")} = (2^n - 2n)x$$

where does the 2n term come from? Describe precautions/concerns that must be taken when amplifying DNA using PCR.

4. Restriction enzymes cleave double stranded DNA to give two possible products: “sticky ends” or “blunt ends”. When would you use each technique and why?

Chemical Biology Problem set #4

1. In class we discussed an early paper from the Dervan lab in which the polyamide ImPyPy was shown to bind the sequence 5′-(W)G(W)C(W)-3′ (where W = A or T) in both possible orientations.
   (a) Which technique, footprinting or affinity cleavage, is best suited to determine the binding orientation of a ligand for DNA?
   (b) Draw a picture illustrating what the gel would look like had this molecule bound in only one orientation.

2. Consider the structures of the four Watson Crick base pairs that form in duplex DNA.
   (a) Draw the structures of these four base pairs.
   (b) Label which atoms lie in the DNA major groove (assume B form DNA) and which atoms lie in the minor groove.
   (c) Label all the potential hydrogen bond donating atoms and all the hydrogen bond accepting atoms in each of the two grooves of all four base pairs.
   (d) Draw structures illustrating how these four base pairs and successfully discriminated by polyamide-like molecules.

3. Draw structures and arrows illustrating a likely mechanism for the C+T-reaction in Maxam-Gilbert sequencing (the hydrazine reaction).

4. The products of a DNA affinity cleavage or MPE-Fe footprinting reaction are often compared with those that result from either a Maxam-Gilbert or Sanger sequencing reactions. Think about the products that are formed in each case and the fact that high-resolution sequencing gels (which is how the products are separated) can often distinguish products that differ by a single phosphate on the 3′ or 5′ end.
   (a) Would you expect the products of an affinity cleavage or MPE-Fe footprinting reaction to migrate alongside the products of a Maxam-Gilbert sequencing reaction? Why or why not? [Please use structures to support your answer.]
   (b) Would you expect the products of an affinity cleavage or MPE-Fe footprinting reaction to migrate alongside the products of a Sanger sequencing reaction? [Please use structures to support your answer.]

Chemical Biology Problem set #5

1. In the manuscript "*Highly Sensitive in Vitro Selections for DNA-Linked Synthetic Small Molecules with Protein Binding Affinity and Specificity*", approximately 10^3-10^7 DNA-linked glutathione amide molecules were combined with a 100- to 10^6-fold molar excess of a DNA-linked negative control, and the resulting mixtures

were selected for binding to GST-linked agarose beads. The selection enriched as few as 10^4 copies of the DNA-linked glutathione by 100- to $>10^4$-fold. The authors claim: "these results demonstrate that selections for modest protein affinities are possible in this format". Without paraphrasing the text in the *JACS* paper, explain why their claim is valid.

2. **Vocabulary**: Please define the following phrases or words.

    a. mutagenic PCR
    b. affinity chromatography
    c. FLAG tag
    d. Molecular breeding
    e. Sequence-space
    f. Effective molarity

3. Consider the DNA Display method described by Halprin & Harbury (PLoS **2004**).

    a. Explain how this method works.
    b. Sometimes the machines that synthesize oligonucleotides make mistakes and delete a base during synthesis. How would this deletion be detected? How would it affect the routing scheme?
    c. The conditions used to route the DNA in this scheme seem pretty harsh – 1.5 M NaCl! What is the likely role of the high salt concentration?
    d. What are three advantages of DNA display over DNA-templated synthesis?
    e. Are there any disadvantages?
    f. What other uses might be imagined for this method?
    g. Explain how this method could be used to prepare 10^{12} distinct small molecules from 30 pmol DNA starting material.
    h. What improvements could you imagine that would further increase the diversity of the pool or the ease with which this method can be applied?

Chemical Biology Problem set #6

1. List three criteria that should be considered when designing a molecule that can recruit two receptors at once (a "chemical inducer of dimerization").
    a. modification must not decrease affinity or specificity
    b. modified molecule must be easy to synthesize
    c. modification should not alter cellular localization

2. Let's say you have synthesized a potential CID, and you are interested in determining if it binds its respective receptors with affinities that are comparable to the wild type molecule.
    a. How would you design and analyze this binding experiment?
    b. How would you determine if binding of the CID to receptor A was altered by the presence of a bound receptor B?
    c. What techniques would you use to monitor binding?

3. In Lin et al. 2000, it was observed that *lacZ* transcription was dependent on the concentration of Dex-Mtx when [Dex-Mtx] ranged between 0.01 and 10 μM.
    a. Should this correlation hold at concentrations much higher than 10 μM? Why or why not?
    b. These authors also observed that addition of a 10-fold excess of Dex did not decrease the extent of lacZ transcription (while a 10-fold excess of Mtx did). What do you think about the authors explanation of the seemingly failed control experiment?
    c. Why is Dex-Mtx a better CID than Dex-FK506?

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4. The authors of Baker et al 2002 describe an interesting strategic choice. They chose to insert a bond to be *cleaved* between Mtx and Dex, and select for molecules that decrease lacZ transcription, rather than inserting a bond to be *synthesized* between Dex and Mtx, and selecting for increased lacZ transcription.
    a. Was this choice wise? What are two advantages and disadvantages of each strategy.
    b. Can this method be extended easily to identify enzymes that do not make or break a bond between the activation and DNA-binding domains?