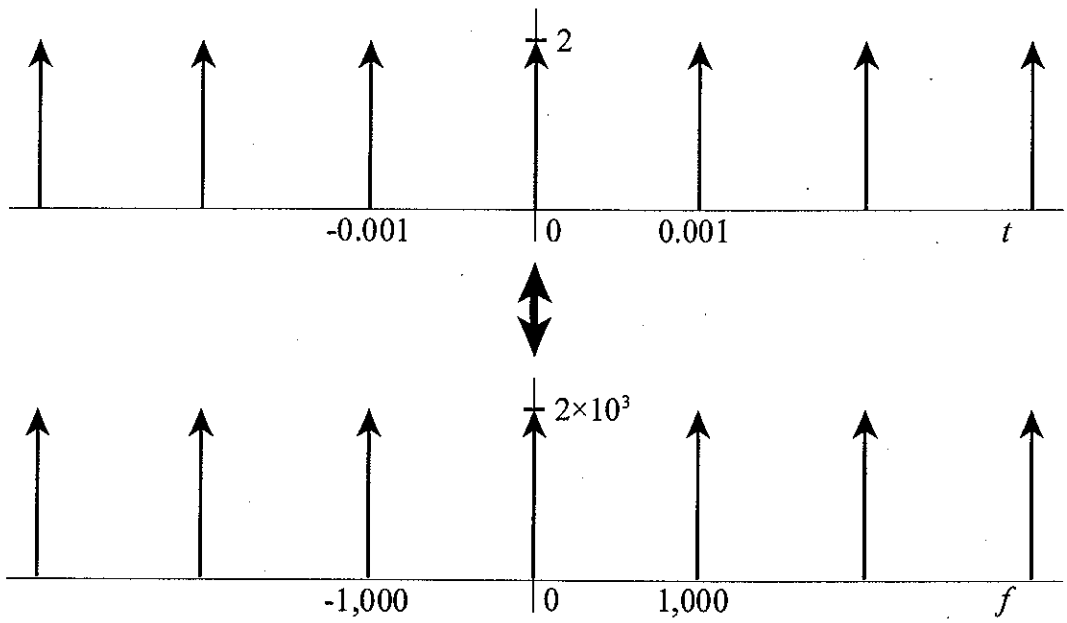
**Crib Analytical Cumulative Exam April 2, 2005**

(a) The temporal comb is given below. Anticipating the final answer I gave the comb function an amplitude of 2. The temporal period is $t^0 = 0.001$ seconds. The Fourier transform of a comb is a comb with an amplitude of $2f^0 = 2\times 1/t^0 = 2\times 10^3$, where the factor of 2 comes from the temporal comb amplitude.



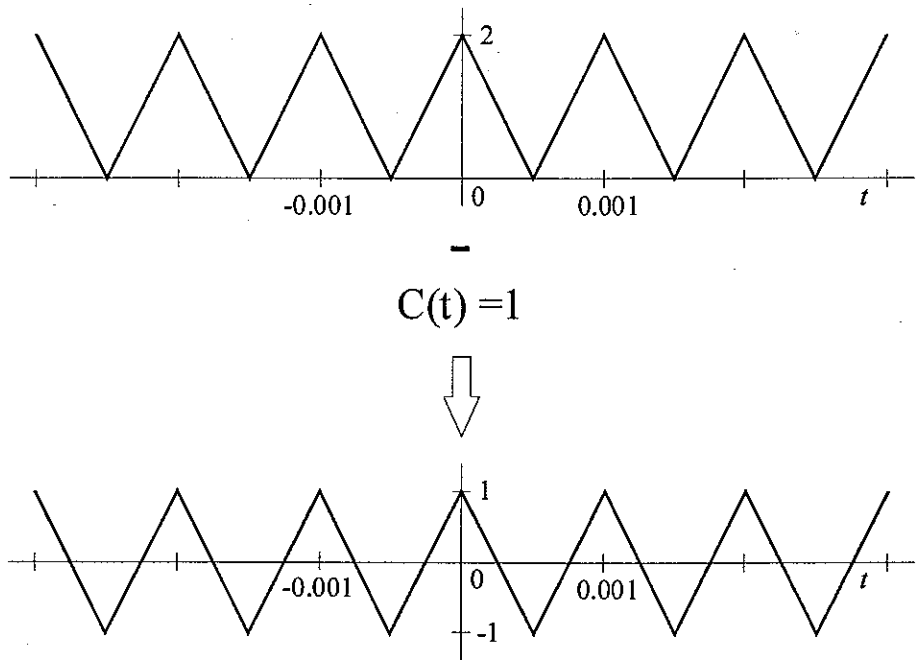

(b) A triangle is generated by convolving two identical rectangles. If $\Delta t$ is the triangle FWHM the width of both rectangles will need to be $\Delta t$. The Fourier transform of a triangle is then the square of the Fourier transform of the rectangle. Note that $\Delta f = 1/\Delta t$.

$$[(1/\Delta t)^{1/2}\,\mathrm{rect}(\Delta t)]\otimes[(1/\Delta t)^{1/2}\,\mathrm{rect}(\Delta t)] \leftrightarrow [(1/\Delta t)^{1/2}(1/\Delta f)\,\mathrm{sinc}(\Delta f)]^2$$

The frequency term reduces to $(1/\Delta f)\,\mathrm{sinc}^2(\Delta f)$.

The triangle needed for convolution with the comb must have a FWHM = 0.0005, thus the two rectangles have widths of 0.0005. In the frequency domain the triangle spectrum is given by $\Delta f = 2\times 10^3$, i.e. $0.0005\,\mathrm{sinc}^2(2\times 10^3)$.

(c) The waveform generated by convolving the temporal comb and triangle is shown below. From this it is seen that a constant of 1 needs to be subtracted from the temporal data. This results in subtracting $\delta(f = 0)$ from the spectrum.




(d) The temporal waveform and its transform can be written as follows.

$$[2\ \mathrm{comb}(10^{-3}) \otimes \mathrm{triangle}(0.0005)] - 1 \leftrightarrow [2\times10^{3}\ \mathrm{comb}(10^{3}) \bullet 0.0005\ \mathrm{sinc}^{2}(2\times10^{3})] - \delta(0)$$

The constant factors in front of the two functions multiply to 1. Since $f = 0$ has an amplitude of 1 and the $\delta(0)$ has an amplitude of -1, the zero frequency component disappears. Also note that the node spacing of the sinc function is twice the frequency spacing of the comb. As a result all of the even harmonics will disappear. The fundamental and odd harmonics have the following amplitudes.

$$f^0 \qquad \left[\frac{\sin\left(\pi f^0/2f^0\right)}{\pi f^0/2f^0}\right]^2 = \frac{4}{\pi^2}$$

$$3f^0 \qquad \left[\frac{\sin\left(\pi 3f^0/2f^0\right)}{\pi 3f^0/2f^0}\right]^2 = \frac{4}{\pi^2}\frac{1}{3^2}$$

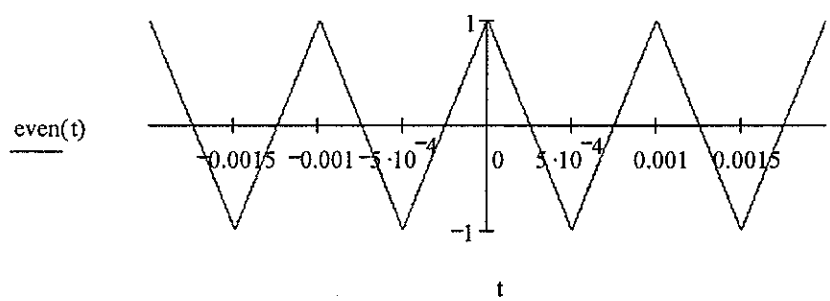
$$5f^0 \qquad \left[\frac{\sin\left(\pi 5f^0/2f^0\right)}{\pi 5f^0/2f^0}\right]^2 = \frac{4}{\pi^2}\frac{1}{5^2}$$

The spectrum then consists of an infinite train of impulse functions.

$$\Phi(f)=\frac{4}{\pi^2}\left[\cdots+\frac{\delta\left(-5\times10^3\right)}{5^2}+\frac{\delta\left(-3\times10^3\right)}{3^2}+\frac{\delta\left(-1\times10^3\right)}{1^2}+\frac{\delta\left(1\times10^3\right)}{1^2}+\frac{\delta\left(3\times10^3\right)}{3^2}+\frac{\delta\left(-5\times10^3\right)}{5^2}+\cdots\right]$$

This expression was implemented in Mathcad as a Fourier cosine series to demonstrate that it is correct.

$$i := 1,3..50 \qquad \text{even}(t) := \frac{4\cdot 2}{\pi^2}\cdot\left(\sum_i \frac{\cos\left(2\cdot\pi\cdot i\cdot 10^3\cdot t\right)}{i^2}\right)$$




(e) The shift theorem states that for a temporal shift of t' the Fourier transform is modified as shown below.

$$F(t-t')\leftrightarrow\Phi(f)e^{-i2\pi ft'}$$

The odd triangular wave is shifted from the even triangular wave by $t' = t^0/4$. The exponential needs to be evaluated for odd harmonics, $nf^0 = 1/t^0$. This results in $\Phi_{odd}(f) = \Phi_{even}(f)\times e^{-in\pi/2}$.

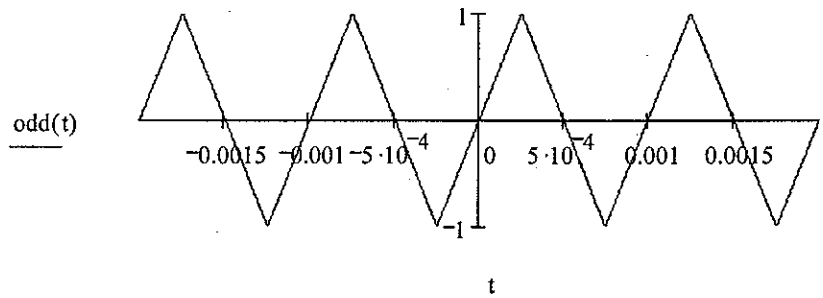
| $n$ | $-7$ | $-5$ | $-3$ | $-1$ | $1$ | $3$ | $5$ | $7$ |
|---|---|---|---|---|---|---|---|---|
| $e^{in\pi/2}$ | $e^{i7\pi/2}$ | $e^{i5\pi/2}$ | $e^{i3\pi/2}$ | $e^{i\pi/2}$ | $e^{-i\pi/2}$ | $e^{-i3\pi/2}$ | $e^{-i5\pi/2}$ | $e^{-i7\pi/2}$ |
| *value* | $i$ | $-i$ | $i$ | $-i$ | $i$ | $-i$ | $i$ | $-i$ |

The result of the temporal shift is to create a spectrum composed of sines having an alternating amplitude with that of the fundamental, $f^0$, being -1.
This expression was implemented in Mathcad as a Fourier sine series to demonstrate that it is

$$\Phi(f)=\frac{4i}{\pi^2}\left[\cdots+\frac{\delta\left(-5\times10^3\right)}{5^2}-\frac{\delta\left(-3\times10^3\right)}{3^2}+\frac{\delta\left(-1\times10^3\right)}{1^2}-\frac{\delta\left(1\times10^3\right)}{1^2}+\frac{\delta\left(3\times10^3\right)}{3^2}-\frac{\delta\left(-5\times10^3\right)}{5^2}+\cdots\right]$$

correct.

$$i := 1,3..50 \qquad odd(t) := \frac{4\cdot 2}{\pi^2}\cdot\left[\sum_i (-1)^{\frac{i-1}{2}}\cdot\frac{\sin\left(2\cdot\pi\cdot i\cdot 10^3\cdot t\right)}{i^2}\right]$$



# Biochemistry Cumulative Examination
# 04/02/05
# Title: Enzymology
# SubTitle: Proteolysis

---

**1 (15 pts)** Provide a brief general definitions (with aid of illustrations, if necessary) for the following terms describing enzyme catalysis:

*i.* 'kinetic mechanism'

**sequence of binding/dissociation events in the course of an enzymatic process**

*ii.* 'chemical mechanism'

**sequence of chemical reactions/steps/transformations involved in an enzymatic process**

*iii.* 'Michaelis complex'

**enzyme-substrate complex**

*iv.* 'suicide inhibitor'

**a mechanism-based inhibitor, which bind to an enzyme acytive site in an irreversible manner.**

*v.* 'zymogen'

**inactive enzyme precursor**

**2 (15 pts)** Proteases are often key players in a wide range of biological processes and are therefore common targets for therapeutic intervention Name key proteases (or protease classes) involved in the following processes and classify them as serine, cysteine, aspartic proteases, or metalloproteases:

*i.* Angiogenesis (matrix remodeling)

**matrix metalloproteinases (MMP-2 and MMP-9): metalloproteases**

*ii.* Apoptosis

**caspases: cysteine proteases**

*iii.* Neurodegeneration in Alzheimer's disease

**β-secretase (BACE): aspartic protease**

*iv.* HIV replication

**HIV-1 protease: aspartic protease**

*v.* Blood clotting

**Factor X, Factor XI, Thrombin, Plasmin: serine proteases**

**3 (20 pts)** Describe the <u>chemical mechanism</u> of hydrolysis of specific peptide bonds in chymotrypsin by answering the following questions:

a. Describe substrate binding, including the role and chemical nature of the <u>"specificity pocket"</u> in chymotrypsin and trypsin, as well as other binding interactions between a peptide substrate and the enzyme.

**A specificity pocket, determines specificity of protease (chymptrypsin cleaves after an aromatic residue, and trypsin cleaves after a Lys or Arg). Peptide substrate bind an active site of protease in an extended chain (β-strand-like) topology.**

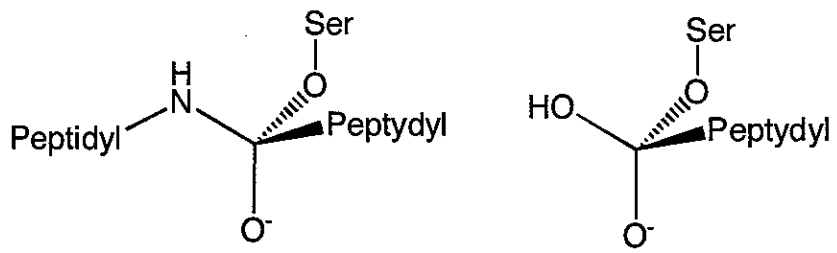
b. Draw the structure of the catalytic triad found in all serine proteases (including structures of the amino acid side chains and hydrogen bonds. What is the role of each member of the catalytic triad in the reaction?

**A catalytic triad scheme should show Asp carboxylate interacting with His imidazole proton, and His basic nitrogen interacting with hydroxyl of Ser. Asp assists in proton shuttling, His is a general base, and Ser is a nucleophile.**

c. What is the nucleophile that attacks the carbonyl group in acylation? In deacylation?

**Ser hydroxyl is a nucleophile on acylation, water is a nucleophile in deacylation**

d. Draw the structures of each of the tetrahedral intermediates in the reaction. Indicate the leaving group in each structure.




**Leaving groups: Peptydyl-NH (Peptydyl-$NH_2$) and Ser-O (Ser-OH), respectively**

e. What is the role of the "oxyanion hole" in the mechanism?

**It stabilizes the tetrahedral transition states, by supplying H-bond donors to the $O^-$ atom (oxyanion)**

**4 (20 pts)** A substantial residual activity of trypsin is maintained even when the catalytic triad is absent. (($k_{cay}/k_{uncay} \approx 10^3$ *JACS* **1992**, *114*, 1784). Provide a plausible explanation for this observation.

**A trypsin, devoid of a catalytic triad , still possesses a specificity pocket, an "oxyanion hole" and other features allowing the catalysis to proceed due to favorable electrostatic and entropic factors**

**5 (10 pts)** In 1954, while examined reaction of chymotrypsin with *p*-nitrophenyl acetate, B. S. Hartley and B. A. Kilby, noted that the release of *p*-nitrophenol involved an initial "burst", equal in magnitude to the concentration of the enzyme. Provide a plausible explanation for that observation.

**Kinetic burst occurs due to fast formation of the acyl-enzyme intermediate, followed by slow hydrolysis.**

**6 (10 pts)** A major difficulty in investigating the properties of the pancreatic serine proteases is that these proteases being proteins themselves are self-digesting. This problem is less severe, however, for solutions of chymotrypsin than it is for solutions of trypsin or elastase. Explain.

**Substrate regions for tryptic self-digestion are more likely to occur on the surface of the enzyme**

**7 (10 pts)** The protease from the human immunodeficiency virus possesses a catalytic diad of two aspartate residues (one residue in each of two identical domains: Asp-25, $pK_a = 3.3$, and Asp-25', $pK_a = 5.3$. In the first step of a peptide bond hydrolysis, one aspartate acts as a base, and the other aspartate acts as an acid. The resulting tetrahedral intermediate is <u>not</u> covalently bound to the enzyme. Write a mechanism for this protease.

**A detailed mechanistic description of an aspartic protease hydrolysis can be found at:**
**http://cgat.ukm.my/protease/asparticmech.html**

Inorganic Cumlative Exam
April 2, 2005

Answers

1. $O_3 + Br^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} BrOOO^-$

Given that $BrOOO^-$ is a steady state intermediate.

$BrOOO^- + H^+ \xrightarrow{k_2} HOBr + O_2$

$BrOOO^- + H_2O \xrightarrow{k_3} HOBr + O_2 + OH^-$

$HOBr + Br^- + H^+ \xrightarrow{fast} Br_2 + H_2O$

$H^+ + OH^- \xrightarrow{fast} H_2O$

16 pts (a) overall rx is: $O_3 + 2Br^- + 2H^+ \longrightarrow Br_2 + O_2 + H_2O$

Acid increases the reaction rate (step 2), but acid is consumed overall. Therefore the rx is not acid catalyzed, but rather is (2) acid assisted.

30 pts (b) Since $BrOOO^-$ is a steady-state intermediate:

$$\frac{d[BrOOO^-]}{dt} = k_1[O_3][Br^-] - k_{-1}[BrOOO^-] - k_2[H^+][BrOOO^-] - k_3[BrOOO^-] = 0$$

$$[BrOOO^-]_{ss} = \frac{k_1[O_3][Br^-]}{k_{-1} + k_2[H^+] + k_3}$$

$$\frac{d[HOBr]}{dt} = (k_2[H^+] + k_3)[BrOOO^-]_{ss} = \frac{(k_2[H^+] + k_3)k_1[O_3][Br^-]}{k_{-1} + k_2[H^+] + k_3}$$

and $$\frac{d[Br_2]}{dt} = \frac{d[HOBr]}{dt} = \frac{k_1(k_2[H^+] + k_3)[O_3][Br^-]}{k_{-1} + k_2[H^+] + k_3}$$

and from the stoichiometry

$$-\frac{d[O_3]}{dt} = \frac{d[Br_2]}{dt} = \frac{k_1(k_2[H^+] + k_3)[O_3][Br^-]}{k_{-1} + k_2[H^+] + k_3}$$

22 pts 1 (c) A steady-state intermediate is a species with negligible concentration that forms and decays during the reaction. Its presence is dictated by the experimental rate expression. Although it does not have appreciable concentration, it represents an energy minimum relative to the transition state. The transition state is an energy maximum. It's composition is dictated by the rate expression. In this case there are two transition states one has the composition $BrO_3H \cdot nH_2O$ and the other has the composition $(BrO_3)^- \pm nH_2O$.

2.

$$CH_3CHO \xrightarrow{k_1} CH_3\cdot + \cdot CHO \quad (1)$$

$$CH_3\cdot + CH_3CHO \xrightarrow{k_2} CH_4 + \cdot CH_3CO \quad (2)$$

$$\cdot CH_3CO \xrightarrow{k_3} CH_3\cdot + CO \quad (3)$$

$$2\,CH_3\cdot \xrightarrow{k_4} C_2H_6 \quad (4)$$

8 pts (1) The chain centers are $CH_3\cdot$ and $\cdot CH_3CO$ ($\cdot CHO$ is not a chain center because it does not propagate the reaction)

8 pts (2) Steps 2 and 3 generate $CH_4$ and CO, which are the main reaction products. The rx is $CH_3CHO \rightarrow CH_4 + CO$. These are termed the chain-propagating steps.

8 pts (3) Minor amounts of $C_2H_6$ are formed and $\cdot CHO$ must also form a minor product(s), perhaps HCHO. Step 1 is the chain initiating reaction. Step 3 is the chain-termination.

Inorg. Chem, April 2, 2005

8 pts 2. (4) 1st example of a chain reaction was the reaction of $H_2(g) + Br_2(g) \rightarrow 2HBr(g)$, where the chain centers are H• and Br•.

100 pts possible

# Organic Cumulative Exam

**April 2nd**

**I.1 (16 pts)** Provide general definitions (with aid of illustrations, if necessary) for the following terms:

*i.* 'stereoelectronic effects'

**conformational/configurational and reactivity effects, determined by orbital interactions.**

*ii.* 'anomeric effect'

**stereoelectronic effect referring specifically to the anomeric center of the carbohydrates, and related systems, resulting in an axial substitution.**

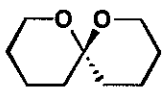
*iii.* 'Bürgi-Dunitz angle'

**angle, observed in crystal structures, which describes approach of a nucleophile to an electrophilic carbon.**

*iv.* 'orbital hyperconjugation'

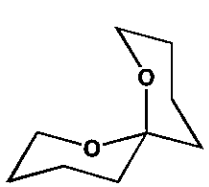
**stabilizing interaction that results from an interaction of donor and acceptor orbitals.**

**I.2 (20 pts)** The conformational equilibrium of spiroketal **1** overwhelmingly favors only one out of three possible arrangements. Which one? Give an explanation by drawing all three contributors.

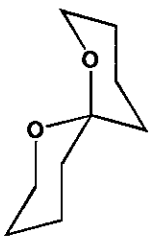


**1**

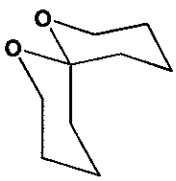
**Conformation A is the most stable one because of the maximally stabilizing orbital interactions (n to σ*)**



**A**

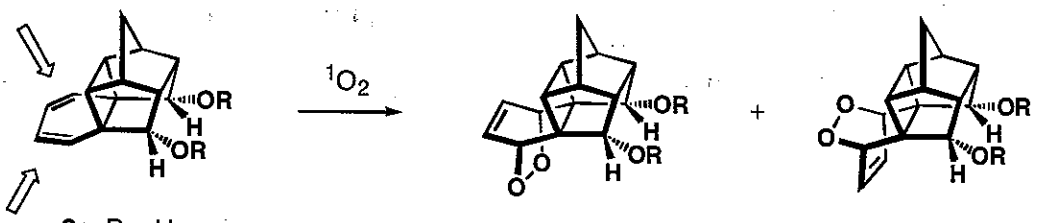


**B**



**C**

**I.3 (14 pts)** Explain the following observations (*Chem. Comm.* **1998**, 1813):




**2a**: R = H
**2b**: R = Ac
**2c**: R = Me

**3a**: 79%
**3b**: 79%
**3c**: 0%

**4a**: 21%
**4b**: 21%
**4c**: 100%

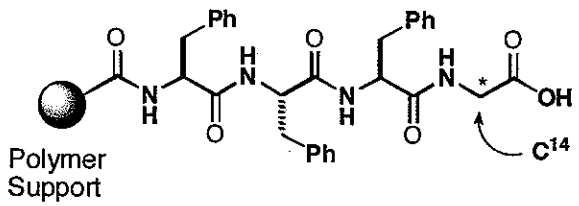
**Hydrogen bond can be invoked to rationalize case a, sterics determines preference in c, stereoelectronics (donor-acceptor interaction: n to $\pi^*$) explains b**

---

**II.1 (15 pts)** Provide names and chemical structures of 20 genetically encoded amino acids. Any of the three ways: a full name, a three-letter or one-letter abbreviations will work. Spelling WILL affect your score. **Extra credit (5 pts)** for the less common 21st one (name + structure)

**Extra credit: selenocysteine**

**II.2 (15 pts)** A few years ago Kahne & Still reported an ingenious method for measuring the rate of peptide bond hydrolysis at room temperature and neutral pH. In this experiment, a $C^{14}$-labelled peptide is attached covalently to a polyacrylamide support and the immobilized peptide is incubated with a buffer. The release of radiolabel into the media is periodically assayed to provide the half-time of hydrolysis at the ambient conditions of seven years (*J. Am. Chem. Soc.* **1988**, *110*, 7529).




a) Why is this paper an important contribution ton the field of Bioorganic Chemistry.

**Background rate for the uncatalyzed cleavage of the peptide bond has been established, so that artificial proteolytic catalysts can be evaluated in their ability to promote peptide bond cleavage.**

b) Several competing processes other than peptide bond hydrolysis might lead to the observed bleed of radioactivity. These are:

*i.* mechanical degradation of the polymeric support;
*ii.* noncovalently adsorbed radioactive peptide;
*iii.* enzymatic contaminants.

Describe the *control experiments* you might perform to distinguish each of these possibilities from the hydrolysis of the terminal peptide bond.

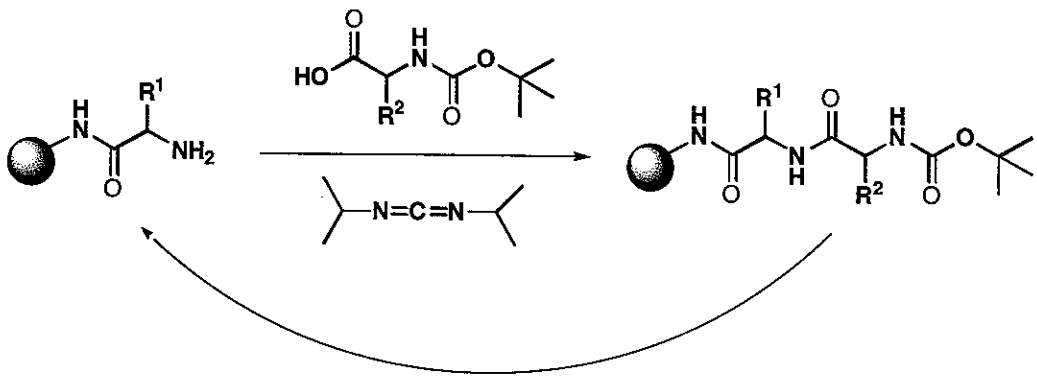
**A lot of reasonable controls would have been accepted. The most obvious ones are:**

**i. Analyze cleavage mixture for presence of only hydrolytic products (Gly, Phe-Gly, Phe-Phe-Gly, etc.)**

**ii. One of the possible controls: synthesize 'cold' peptide, incubate with 'hot' glycine, wash, and compare washings to an identically prepared $C^{14}$ labeled peptide.**

**iii. Denature any enzymatic impurities by using autoclaved buffers, prior to assay.**

**II.3 (20 pts)** R. Bruce Marrifield has been awarded the Nobel Prize in Chemistry in1984 for his development of a straightforward method for obtaining peptides through iterative synthesis. The basic methodology is presented below:




a) **Briefly**, discuss the importance of this development to life sciences.

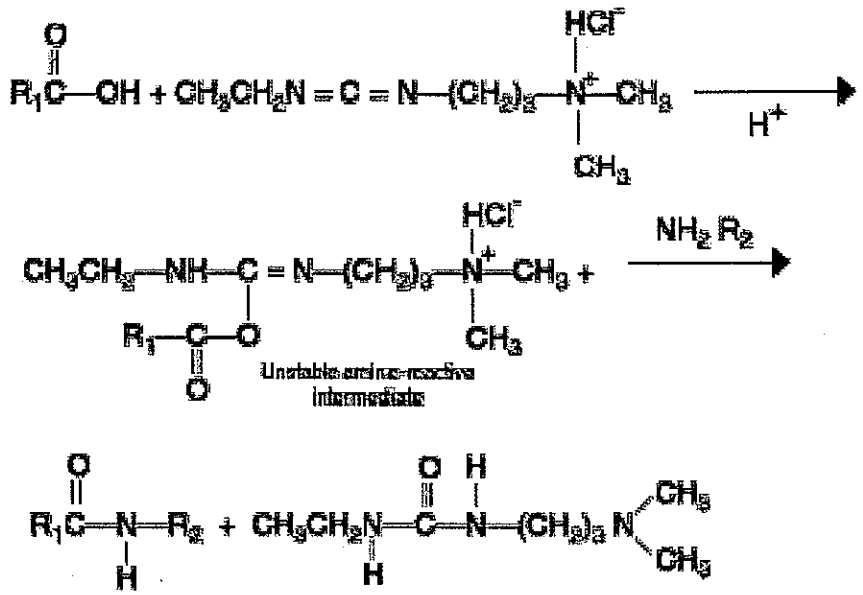
**Access to a wide range of proteinaceous materials (unnatural peptides and proteins, site-specifically labeled natural peptides and proteins) with direct control over their sequence and overall structure.**

b) While the ribosomal polypeptide biosynthesis is in the N to C direction, solid phase peptide synthesis works best in the opposite C to N direction. Why? Provide the mechanistic rationale confirming your answer.

**To minimize epimerization of the growing polypeptide chain: activated carboxylic acid can react with the adjacent amide functionality, leading to a readily epimerizable oxazolidinone intermediate. The latter reaction is suppressed with carbamate protecting groups in the C to N synthesis**

c) Write a detailed mechanism for the carbodiimide-mediated peptide coupling.

**EDC-mediated coupling for example:**



# CRIB (P. chem, April 2, 2005)

1) a) $K = \left(\frac{g_u}{g_f}\right) e^{\overbrace{(\varepsilon_u - \varepsilon_f)}^{\Delta\varepsilon}/kT}$

40 pts.

b) if $g_u = g_f$ then $\Delta\varepsilon \approx -RT\ln K = +13.4\ \text{kJ/mol}$

c) if $\Delta\varepsilon = 0$ then $K = g_u/g_f \cong 0.004$

2) a) $\Delta G = -RT\ln K = +13.4\ \text{kJ/mol}$

30 pts.

b) $\Delta S = -\left(\partial\Delta G/\partial T\right)_P = -\left[+4.2\ \text{kJ/mol} - 13.4\ \text{kJ/mol}\right]/\left[40\text{K} - 20\text{K}\right] \cong +60\ \text{J/K·mol}$

$\Delta H = \Delta G + T\Delta S = \left(\frac{\partial \Delta G/T}{\partial 1/T}\right)_P = R\left(\frac{\partial \ln K}{\partial 1/T}\right) = -RT^2\left(\frac{\partial \ln K}{\partial T}\right)_P$

$\cong +139\ \text{kJ/mol}$

3) a) $\Delta\varepsilon \cong \Delta U \cong \Delta H = +139\ \text{kJ/mol}$ $\quad g_u/g_f \cong e^{+\Delta S/R} \cong 10^{24}$

30 pts.

b) Since $\Delta H >>$ than the $\Delta\varepsilon$ derived in (1.b) that implies that $g_u >> g_f$. This makes physical sense because the unfolded protein has more flexibility (degrees of freedom) than the folded state (although the implied value of $g_u/g_f$ seems to be <u>too</u> large)