Chemistry 5361/8361 Thursday, December 5, 2002

Midterm Exam 3

Please do not open or sign this packet until you are instructed to do so.

Please write all of your answers for this exam this exam packet. Although you may use as many blue books for scratch work as you would like, the blue books will not be collected at the end of the exam or graded. Answer each question in the space provided if you can, but feel free to continue your answer on the back of the page if you need more room. (Please write a note by your answer pointing us to the continuation if you do this.) Feel free to remove the corner staple if this helps you analyze the spectra; you will have the opportunity to re-staple your exam at the end. The exam in this packet is designed to take 1 hour to complete. You will be given 2 hours total to finish the test.

This exam contains two problems, which are split into parts. Many of these parts can be answered independently. *Do not get stuck* on one part and then assume that you will be unable to answer the rest of the question—move on. In addition, partial credit will be given for incorrect but still plausible answers, so *guess* on problems you cannot answer perfectly.

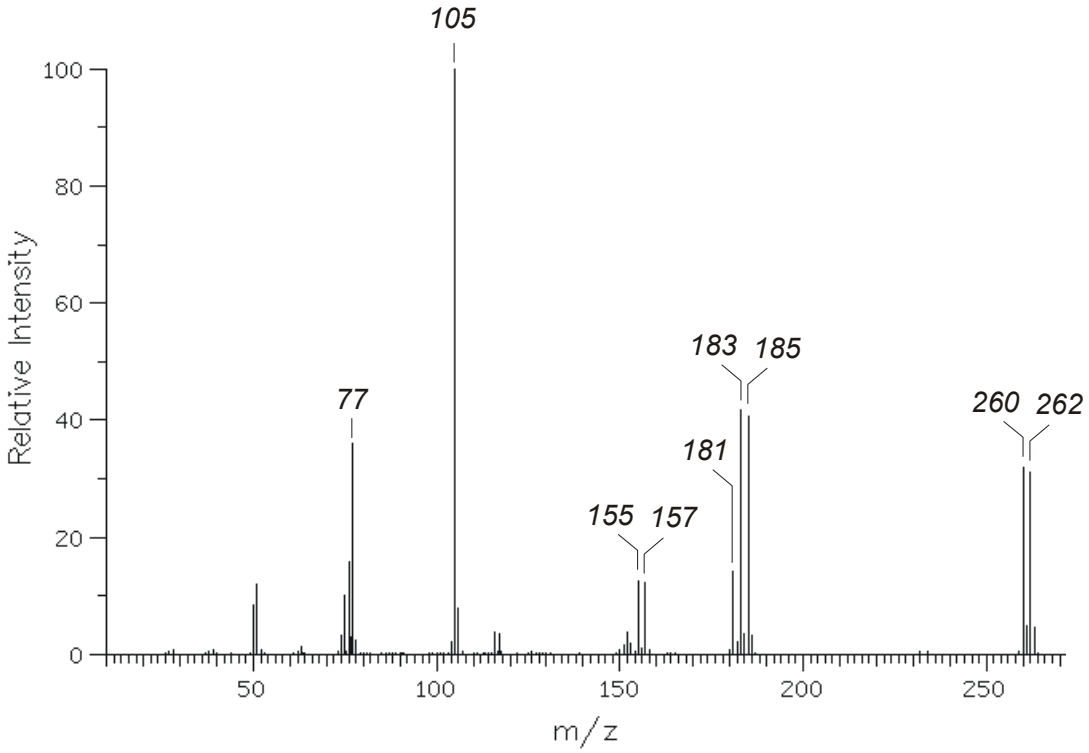
At the end of the 2 hour exam period you will be asked to return your exam to the proctor. (You may, of course, also turn the packet in earlier if you choose.) You are allowed to use any materials you brought with you before the exam. However, we ask that you not bring any materials in or out of the room while you are taking the exam. Please do not take any part of the exam packet with you when you are done; everything will be returned to you after the exams are graded.

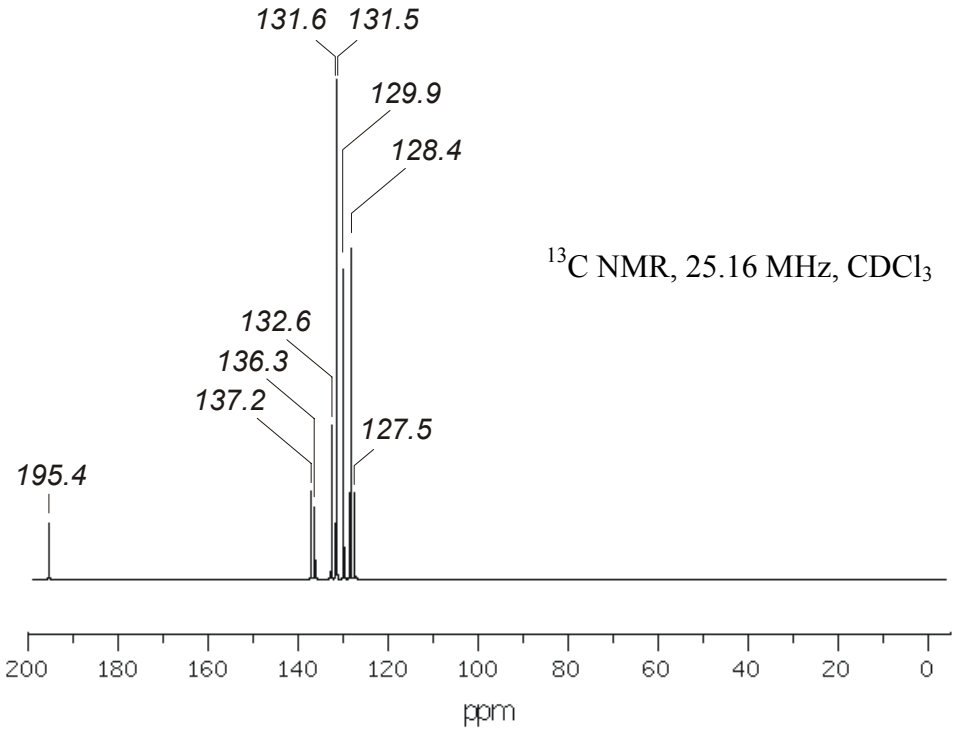
This packet should contain 11 pages, including this one. (The last page contains a chart of isotope ratios and exact atomic masses, and is not part of the graded exam.) Please check to make sure that your packet contains 11 pages before beginning your exam.

Name: _____

Signature: _____

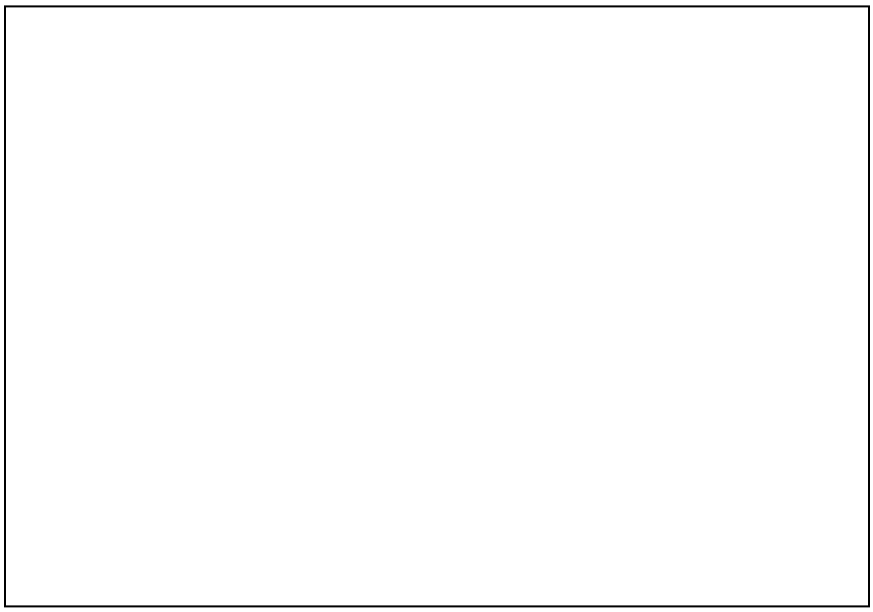
1. (20 pts) As part of a phenyl Grignard reaction gone haywire, one product was isolated that showed the following EI mass spectrum and ^{13}C NMR spectrum:



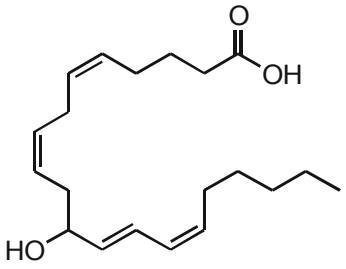


^{13}C NMR, 25.16 MHz, $CDCl_3$

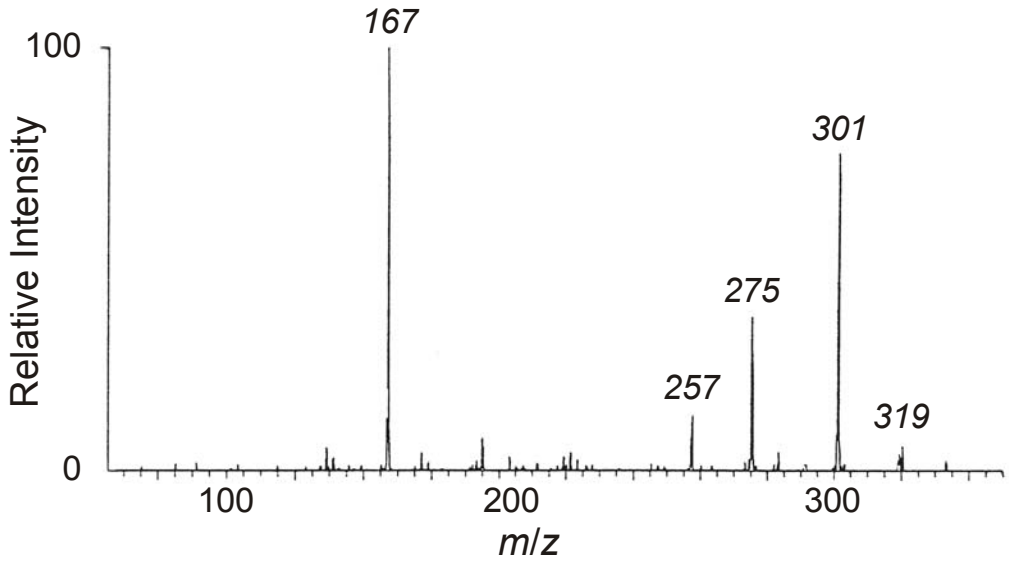
The ^{1}H NMR spectrum showed only overlapping aryl peaks (δ = 7-8 ppm). What was the structure of the unknown product? (If you do not know the answer, *guess*. Partial credit will be given for structural fragments that are found in the correct structure.)



2. The structure of 11-hydroxyeicosatetraenoic acid (**11-HETE**) has been confirmed by negative-ion electrospray MS-MS. In the MS-MS experiment, the ion population with m/z = 319 was selected and subjected to collision-induced dissociation (CID) to generate the spectrum below.



11-HETE
(MW = 320)

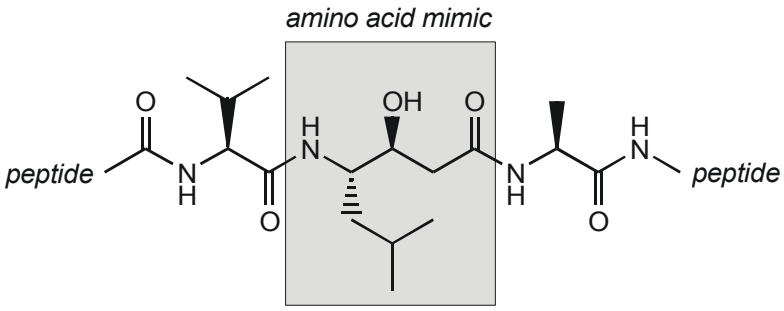


a. (16 pts) Based on the structure for **11-HETE**, provide plausible chemical structures for the ions observed at m/z = 319, 301, 275 and 257. (Draw your answers on the next page.)

m/*z* = **319**	*m*/*z* = **301**
m/*z* = **275**	*m*/*z* = **257**

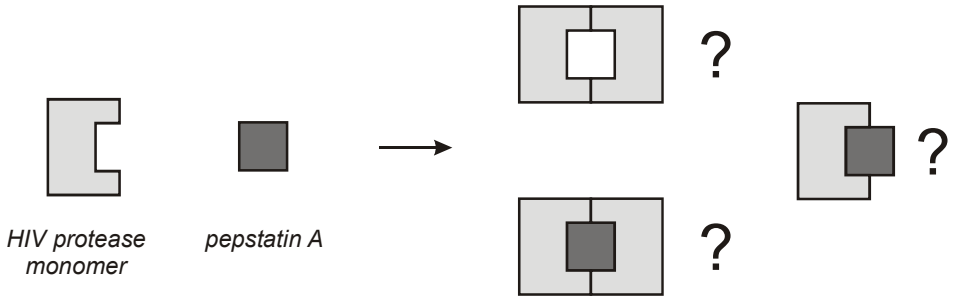
b. (14 pts) The ion observed at *m*/*z* = 167 is produced by a γ-hydrogen rearrangement mechanism. Draw the mechanism for this fragmentation, including the full structures of the parent ion, the *m*/*z* = 167 daughter, and any other fragments generated.

3. HIV protease normally exists as a dimeric protein (with two identical subunits, making it "homodimeric"), and is an attractive target for therapeutic inhibitors that can control HIV infection. One such inhibitor is pepstatin A, a synthetic peptide mimic that binds to the protease active site but cannot be proteolyzed (cleaved) and thus remains noncovalently bound to the enzyme.



pepstatin A

Loo and coworkers at Parke-Davis performed electrospray (ESI) MS experiments to help determine whether pepstatin A bound only to homodimeric HIV protease, or whether it might also bind to the monomeric subunit.

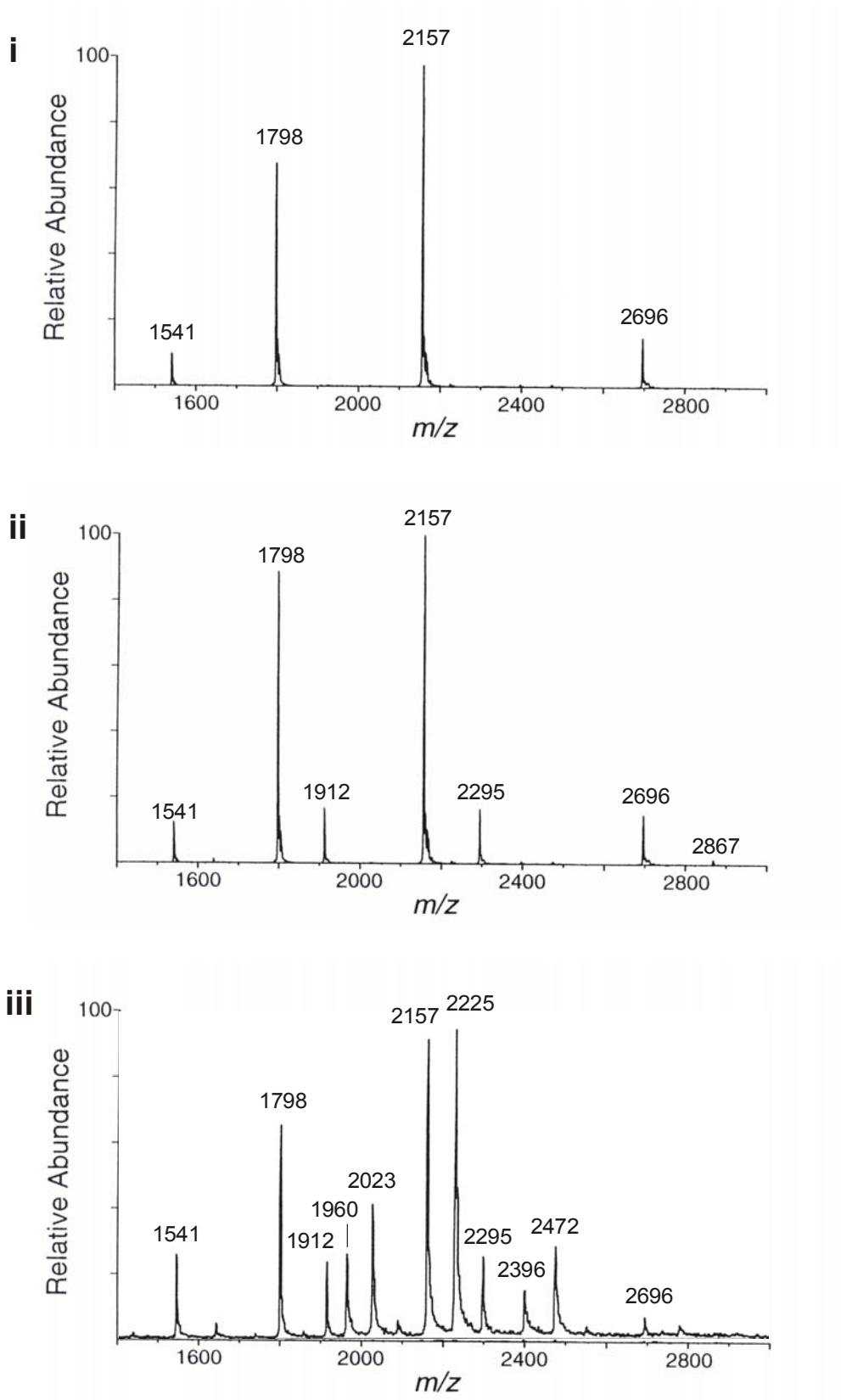


The positive-ion mode ESI mass spectra on the next page were taken from:

(i) a low-concentration solution of HIV protease alone, in which the protein is expected to exist exclusively as a monomeric subunit;

(ii) a low-concentration solution of HIV protease that also contains an equimolar amount of pepstatin A;

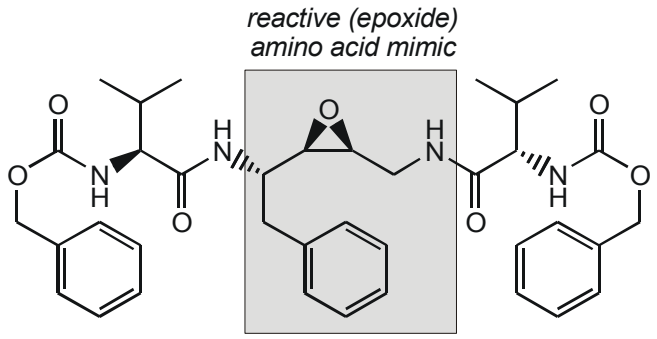
(iii) a high-concentration solution of HIV protease that also contains an equimolar amount of pepstatin A.


i
100
Relative Abundance
2157
1798
1541
2696
1600
2000
2400
2800
m/z
ii
100
Relative Abundance
2157
1798
1912
2295
1541
2696
2867
1600
2000
2400
2800
m/z
iii
100
Relative Abundance
2157
2225
1798
2023
1960
1541
1912
2295
2472
2396
2696
1600
2000
2400
2800
m/z

a. (25 pts) On the chart below, identify each of the components that gives rise to each peak in spectrum (iii). Draw a cartoon representation of each ion (as I did on page 6) and calculate the charge state and mass for each.

***m*/*z* ratio**	cartoon 	**ion charge (*z*)**	**ion mass (*m*)**
1541			
1798			
1912			
1960			
2023			
2157			
2225			
2295			
2396			
2472			
2696			

b. (15 pts) HIV protease inhibitors containing reactive epoxides, such as the molecule below, can permanently and covalently bind to the enzyme's active site.



This is unlike the case of noncovalently bound pepstatin A, which is free to exit the HIV protease active site after binding. Describe a mass spectrometry experiment that might be conducted to determine whether the molecule above is a covalent or noncovalent inhibitor of HIV protease.

c. (10 pts) If the epoxide were a covalent inhibitor of HIV protease, it would end up bound to a specific amino acid residue in the protein. Describe another mass spectrometry experiment that might be conducted to determine which amino acid residue the inhibitor was bound to.