Chemistry 5361/8361 Wednesday, December 3, 2003

**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 in 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.

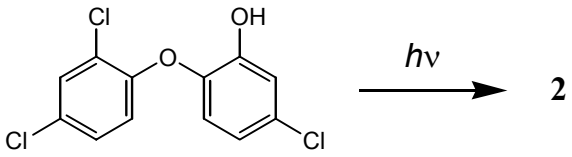
At the end of the 2 hour exam period you will be asked to return your exam to the proctor. (You may, of course, also turn the packet in earlier if you choose.) You are allowed to use any materials you brought with you before the exam. However, we ask that you not bring any materials in or out of the 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 20 pages, including this one. (The last page contains a chart of amino acid structures, and is not part of the graded exam.) Please check to make sure that your packet contains 20 pages before beginning your exam.

**Name:** _____

**Signature:** _____

1. Doug Latch (McNeill Group) studies the photochemical effect of sunlight on chemicals that are released into the environment, with the concern that putatively harmless compounds might be transformed into harmful environmental contaminants by UV light. Recently, Doug showed that the antibacterial agent triclosan (**1**) is degraded by broad UV irradiation into one prominent, unexpected photoproduct. Doug isolated this product and analyzed it by $^1$H NMR, EI-MS and UV-vis spectroscopy.




**1**



a. (6 pts) Based on the observed mass losses in the mass spectrum of **1**, predict the chemical formula for the fragments with *m*/*z* = 218 and 146.

| *m*/*z* = 218 | *m*/*z* = 146 |
|---|---|
| | |

b. (10 pts) Predict the isotopic distribution patterns for the chemical formulae you gave in part (a). (Assume 100% relative intensity for $[M]^{\bullet+}$, and calculate the relative intensities of $[M+1]^{\bullet+}$, etc. to within 5%.) Are the observed isotope distributions for these fragments consistent with your calculations?

***m*/*z* = 218**: (predicted intensity values, in percent)

| M | M + 1 | M + 2 | M + 3 | M + 4 |
|---|---|---|---|---|
| 100 | | | | |

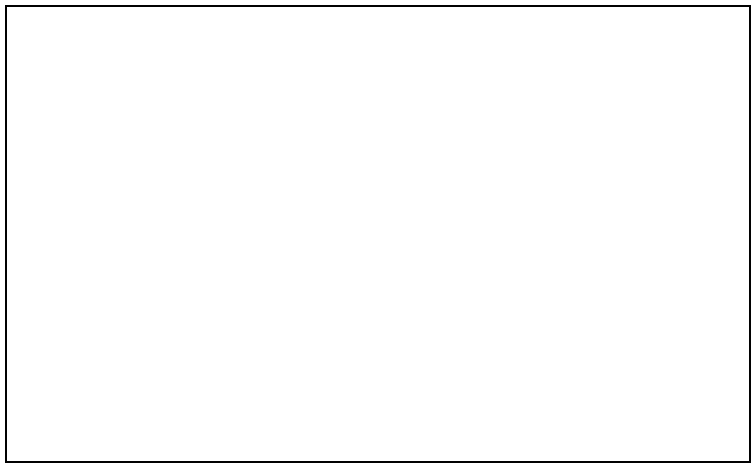
consistent with observed peak intensities? [ ]

***m*/*z* = 146**: (predicted intensity values, in percent)

| M | M + 1 | M + 2 | M + 3 | M + 4 |
|---|---|---|---|---|
| 100 | | | | |

consistent with observed peak intensities? [ ]

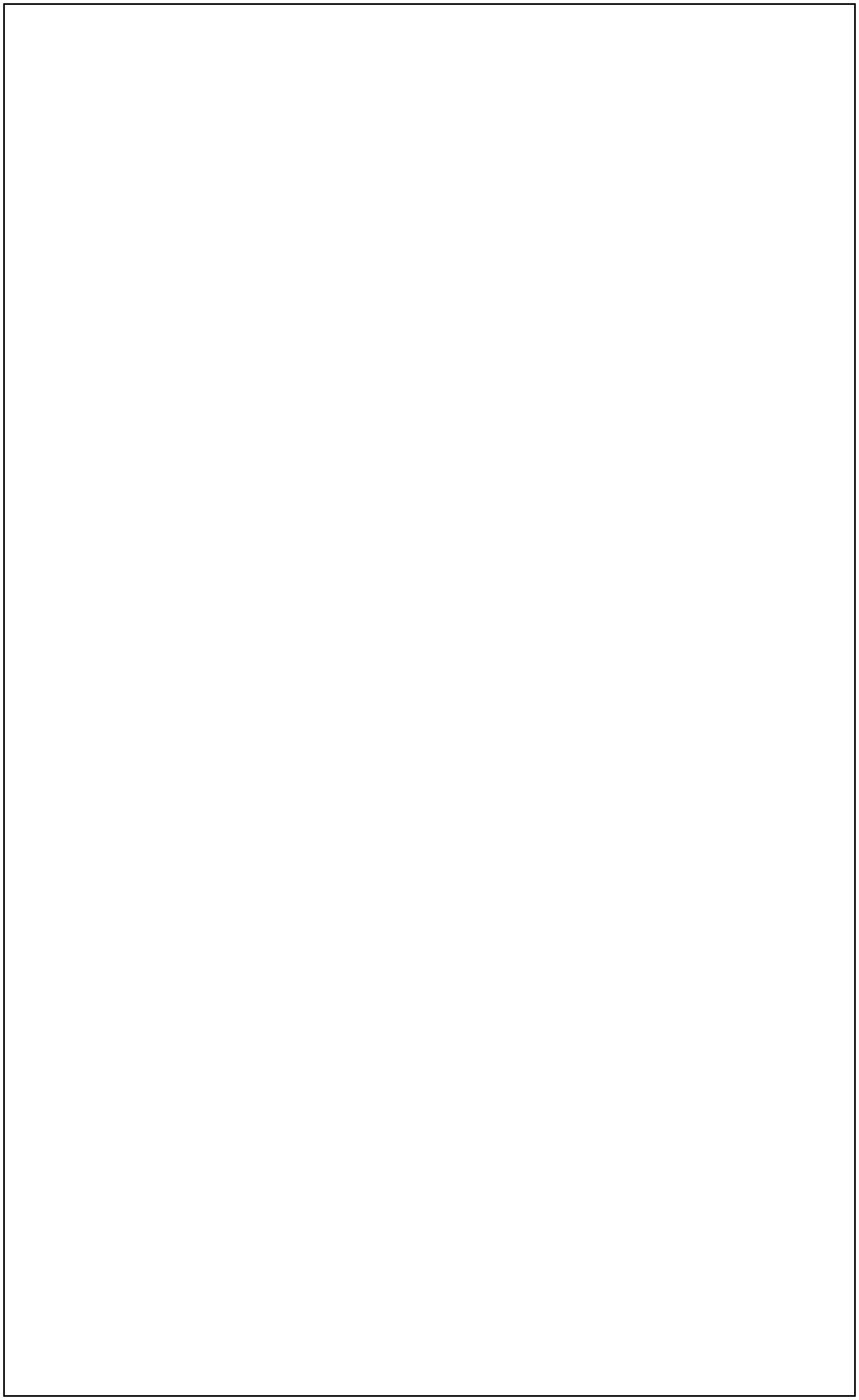
c. (4 pts) What is the structure of **2**?

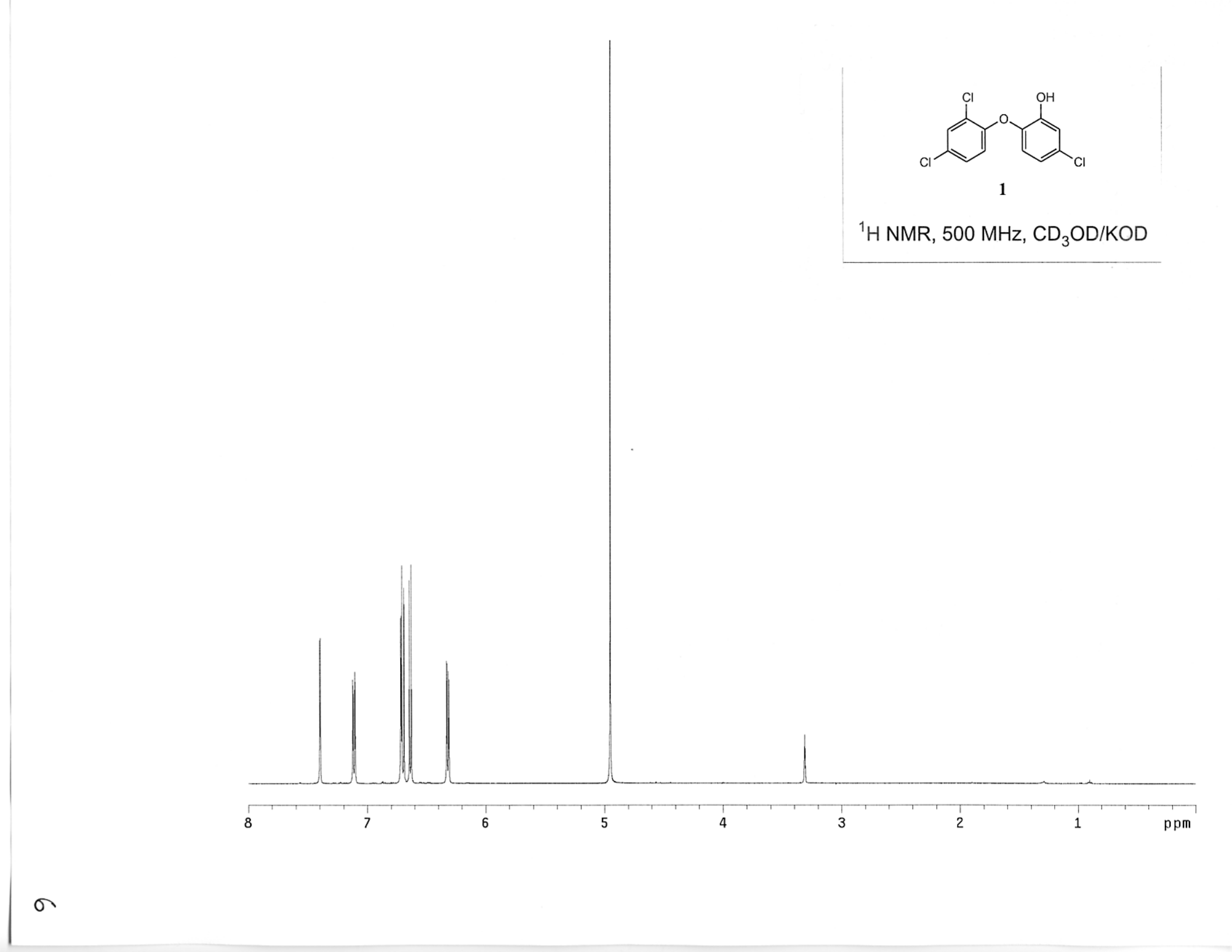


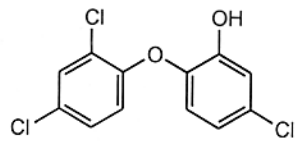
d. (10 pts) The UV-vis $\lambda_{max}$ of **2** appears at a significantly longer wavelength than that of **1**. Explain why in terms of the chemical structures of **1** and **2**.

e. (10 pts) Although Doug demonstrated that triclosan exhibited photochemistry in the lab, his experiments did not directly demonstrate that the same photochemistry occurs in, say, terrestrial rivers. Because triclosan is emitted into waterways in many places, and because photochemistry should occur wherever the sun shines, it would be difficult to quantitatively characterize the natural photodegredation of triclosan. However, it would be possible to artificially spike a river with triclosan in a particular place, and then collect photodegradation products downstream. If the current flow and sun exposure of the river were known, then the conversion rate of triclosan to **2** in the river could be characterized. Importantly, in order for this experiment to work, the spiked triclosan would have to be distinguishable from triclosan that was already present in the water from other sources.

Outline an experimental, mass spectrometric method that could achieve this goal. Assume that you have any chemicals, instruments and manpower that you need to complete your experiment. Be as explicit as you can—what type of instrument will you use? What exact materials would you require? What precise data will you be looking for? How will you collect your sample? Make sure that your experiment can distinguish your triclosan from environmental triclosan.

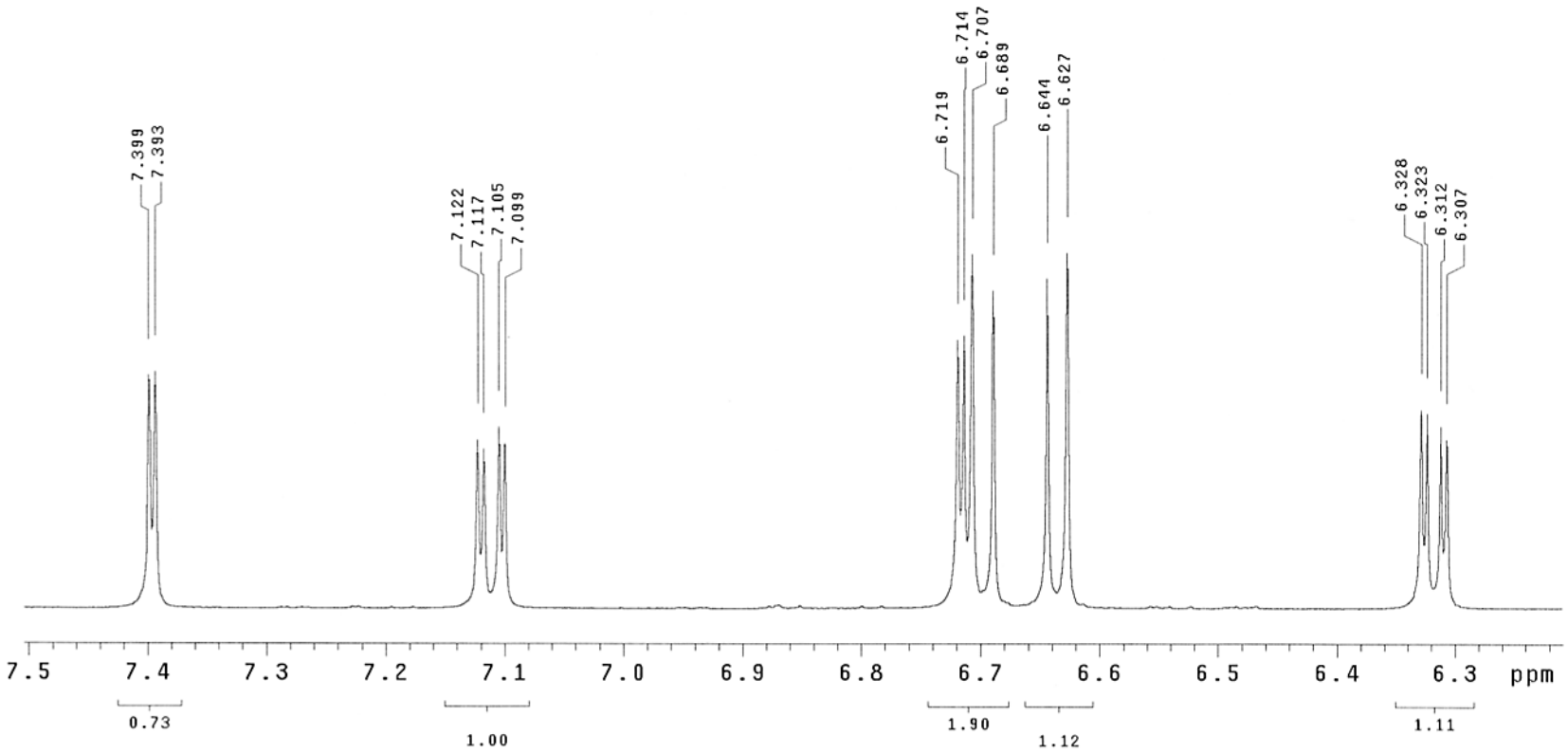



Cl
OH
O
Cl
Cl
1
$^1$H NMR, 500 MHz, $CD_3OD$/KOD
8
7
6
5
4
3
2
1
ppm




**1**

$^1$H NMR, 500 MHz, $CD_3OD$/KOD (*closeup*)

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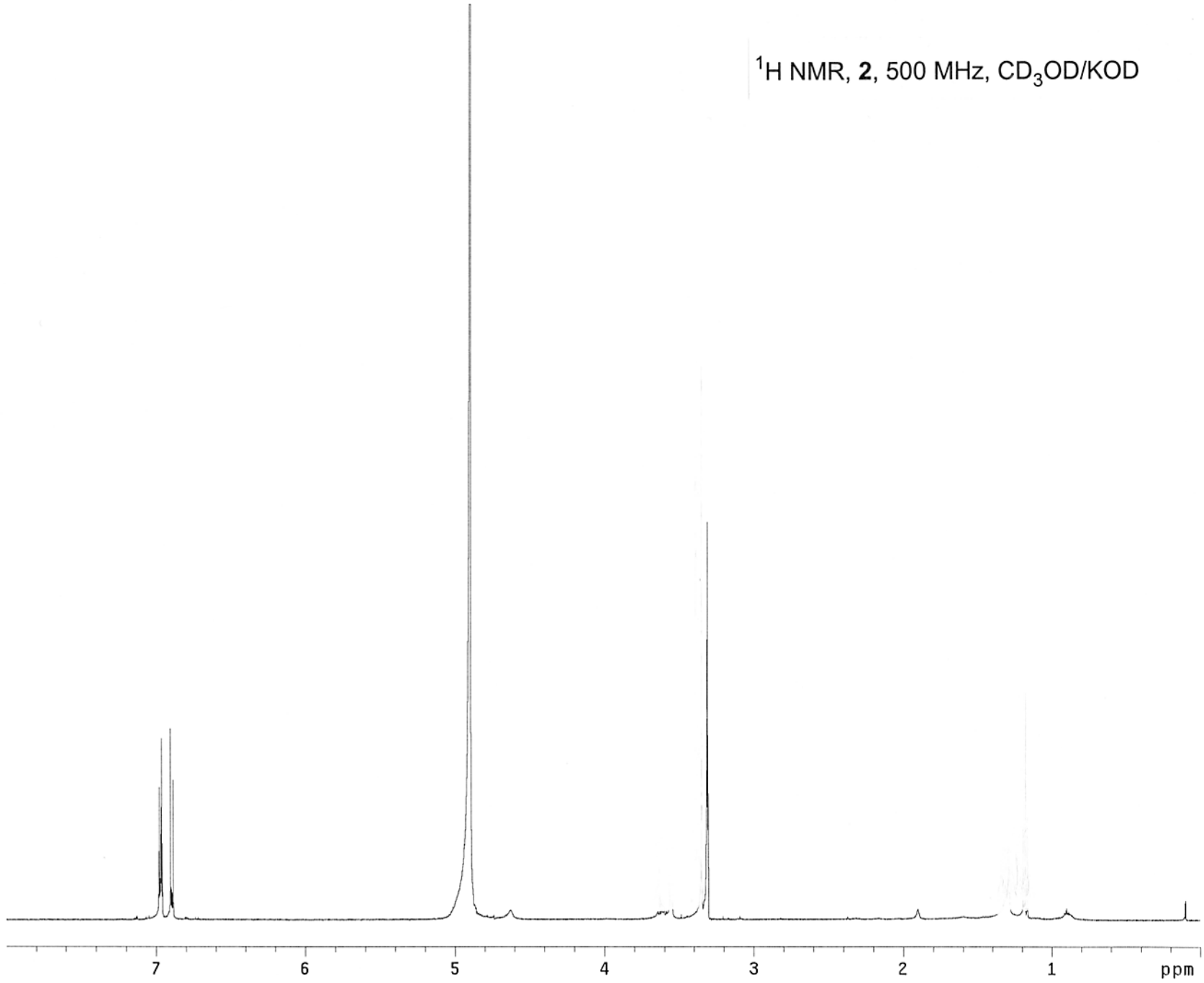
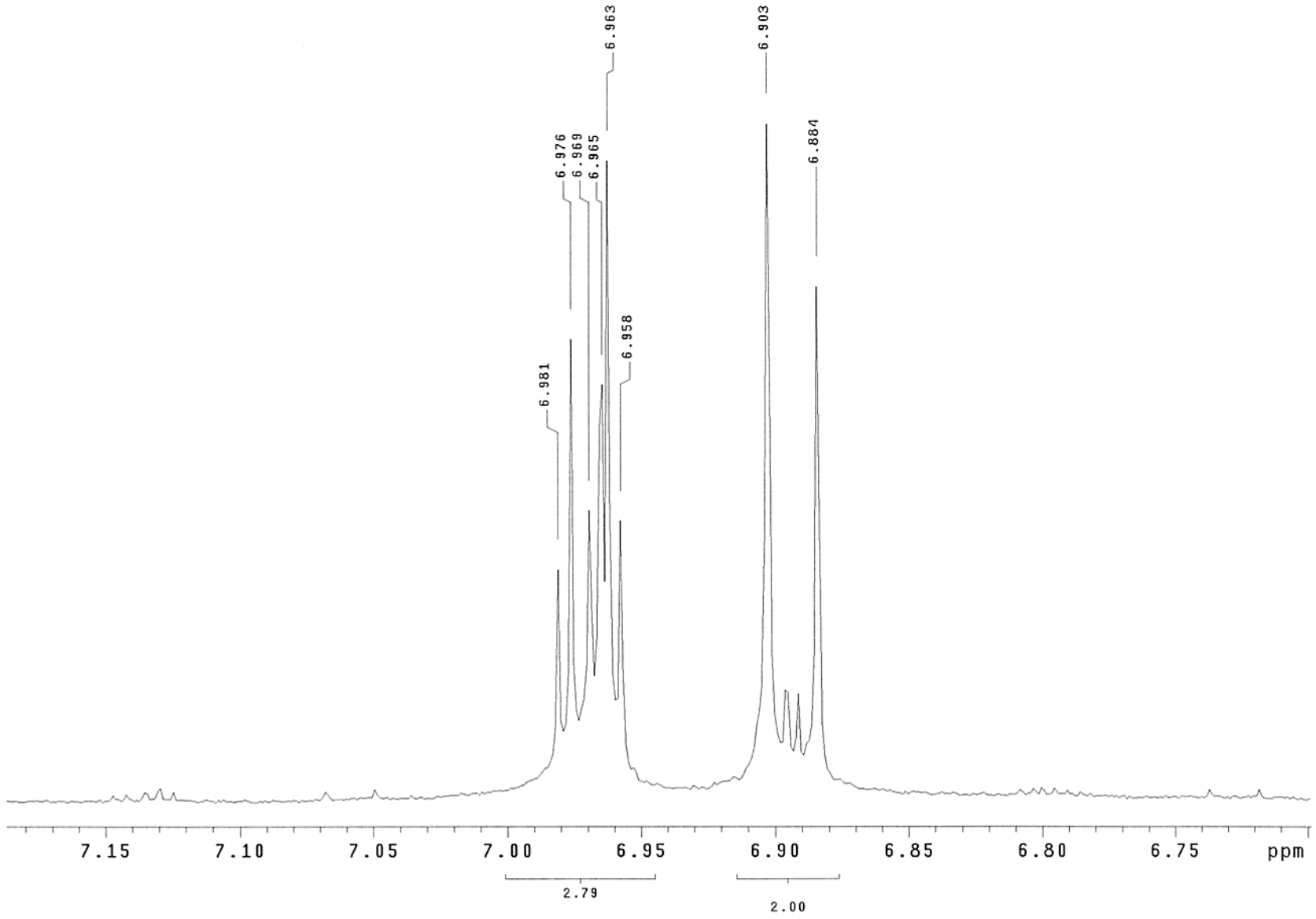

$^1$H NMR, **2**, 500 MHz, $CD_3OD$/KOD
7
6
5
4
3
2
1
ppm

$^{1}$H NMR, **2**, 500 MHz, $CD_3OD$/KOD (*closeup*)



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Instrument :   Instrumen
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Vial Number: 1
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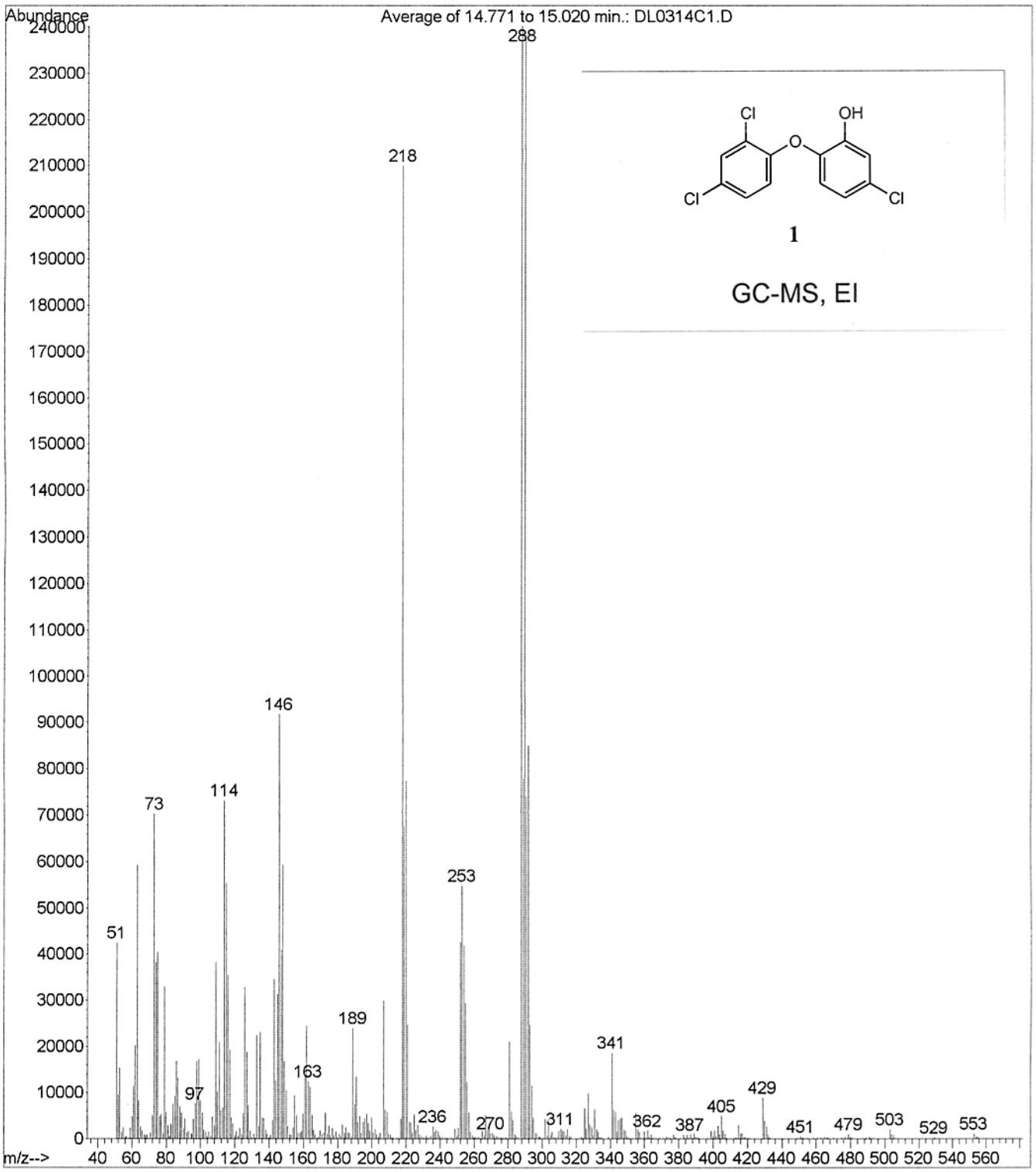

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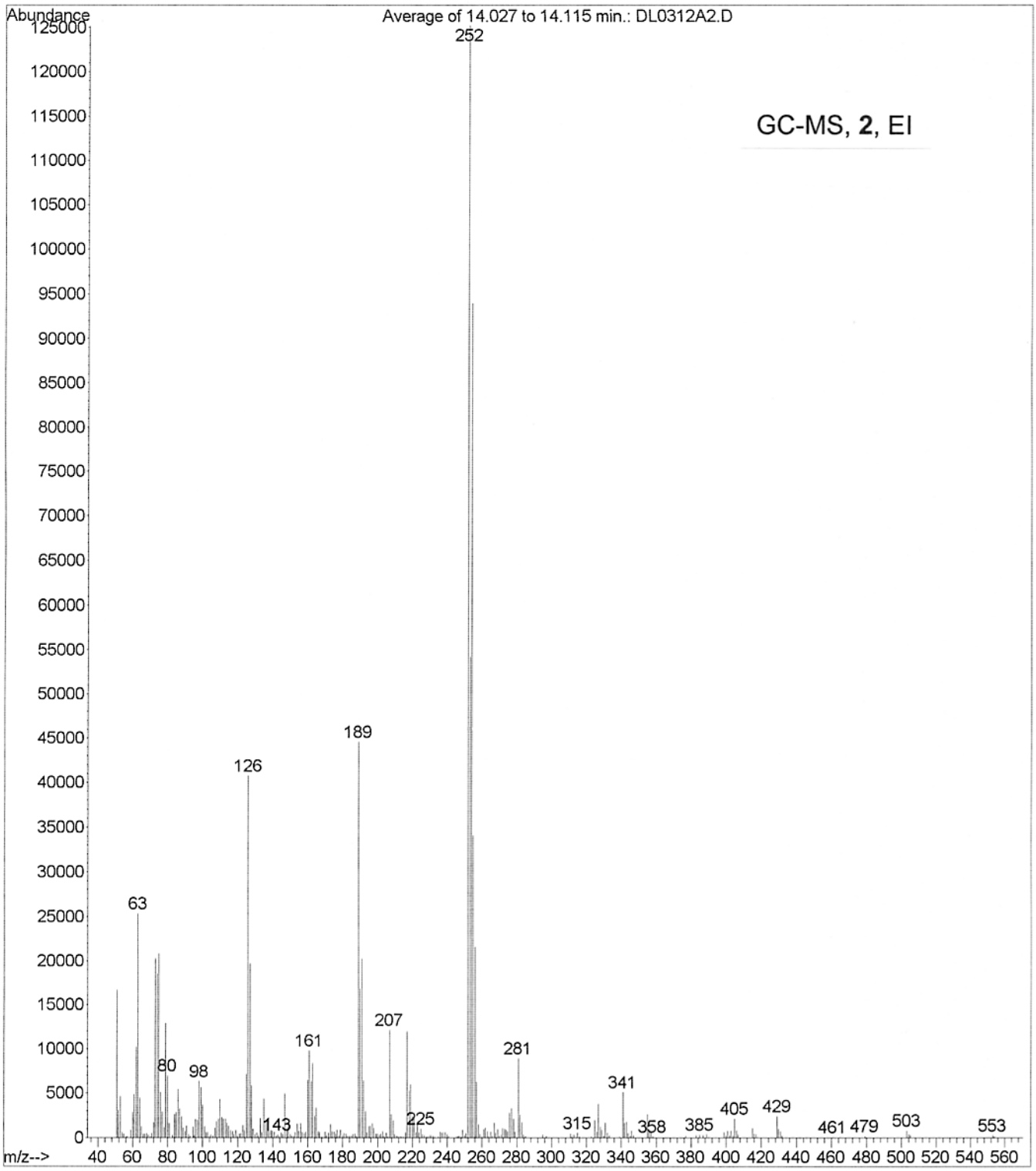
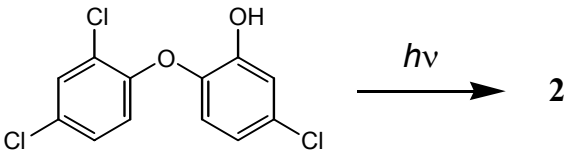
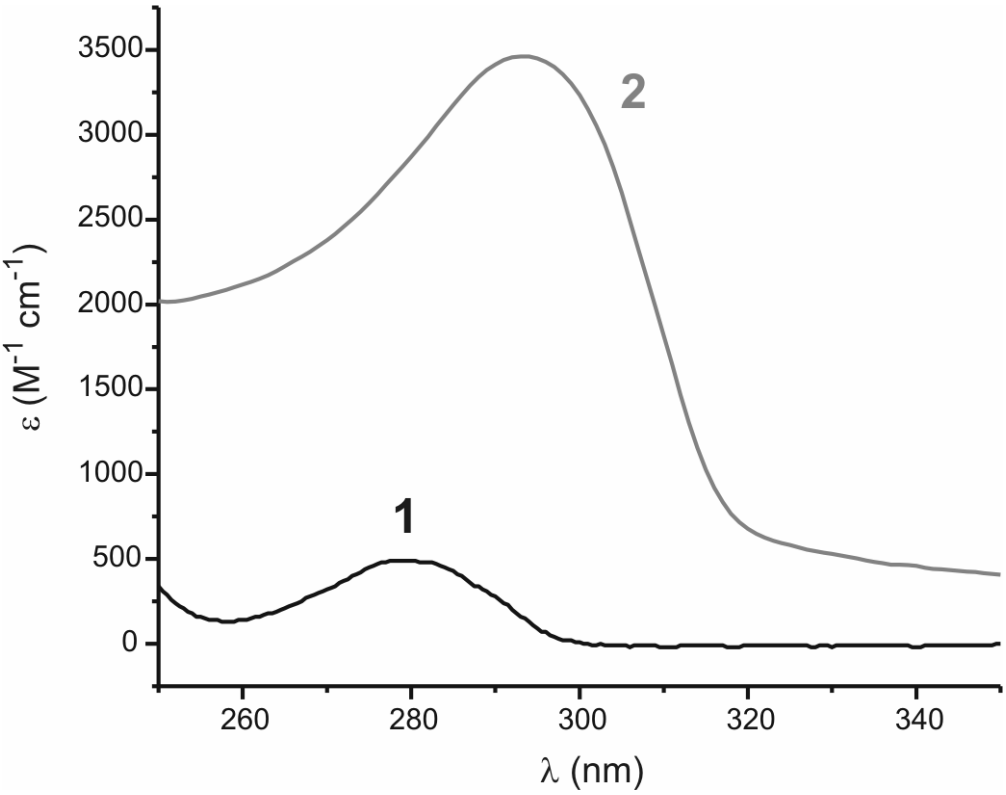
Instrument : Instrumen
Sample Name: III-33, triclosate direct photolysis
Misc Info :
Vial Number: 55



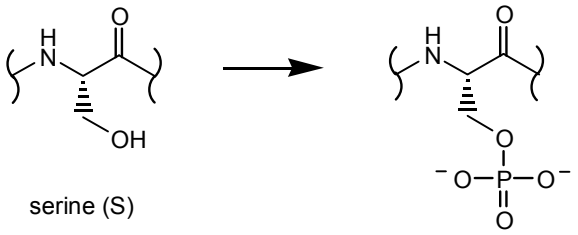
UV-vis spectra, **1** and **2**




**1**



2. Although human proteins are synthesized from only 20 amino acids, these amino acids can be further modified after they have been incorporated into protein structures to provide many more than 20 possible protein building blocks. One common ("posttranslational") modification is phosphorylation, in which alcohol groups are transformed into phosphates.

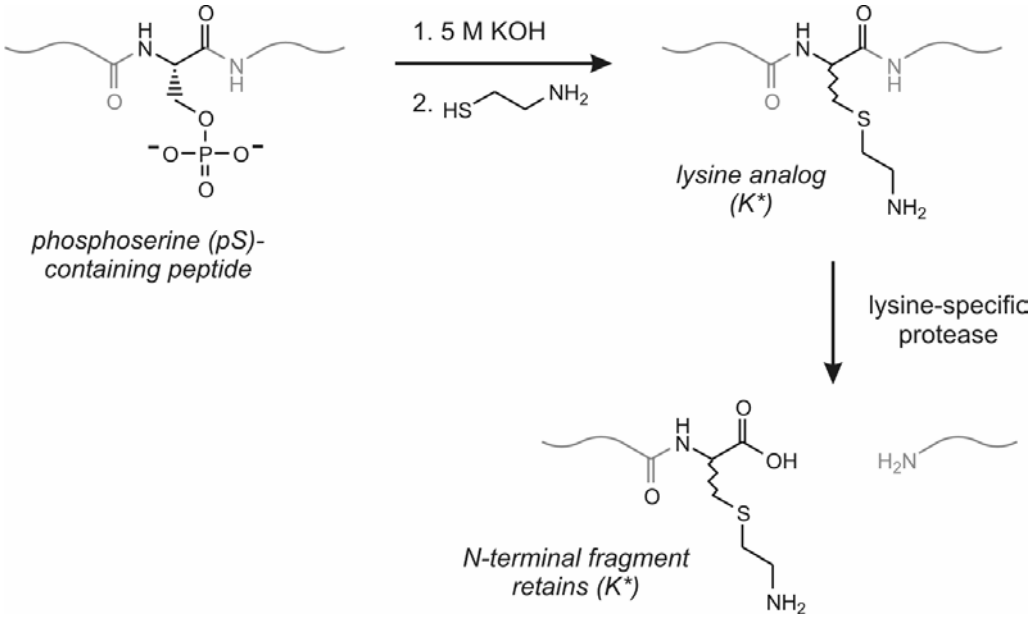


serine (S)

phosphoserine (pS)

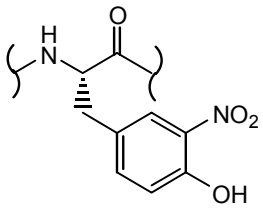
One might expect that mass spectrometry would easily distinguish phosphorylated peptides from unmodified ones. However, the acidity of the phosphate group can suppress positive ion formation via protonation (because it is difficult to neutralize the weak conjugate base), and can make it difficult to observe phosphorylated peptides by MS.

Kevan Shokat and coworkers at UCSF have recently developed a method for identifying phosphorylated serine residues, in which the phosphoserine is first chemically converted into aminoethylcysteine, an analog of lysine.[1] This amino acid (which we will abbreviate K*) is then readily detected by positive-ion mass spectrometry. A unique feature of this method is that a lysine-specific protease is used which cleaves the peptide only if pS has been converted into K*.




[1] Knight, Z. A. et al. *Nat. Biotechnol.* **2003**, *21*, 1047.

Shokat's group investigated whether a signaling peptide was phosphorylated at tyrosine or serine by subjecting it to the protocol outlined above. The peptide had amino acid sequence **YFRPSGF(Y*)D**, where Y* is 3-nitrotyrosine. (The peptide sequence is listed from $–NH_2$ terminus to –COOH terminus.)

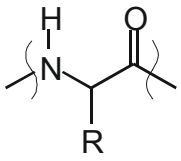



3-nitrotyrosine (Y*)

Fragment masses related to this peptide:

| 1-letter abbrev. | amino acid | fragment mass |
|---|---|---|
| D | aspartic acid | 115.03 |
| F | phenylalanine | 147.07 |
| G | glycine | 57.02 |
| K* | aminoethylcysteine | 146.04 |
| P | proline | 97.05 |
| R | arginine | 156.10 |
| S | serine | 87.04 |
| Y | tyrosine | 163.06 |
| Y* | 3-nitrotyrosine | 208.05 |

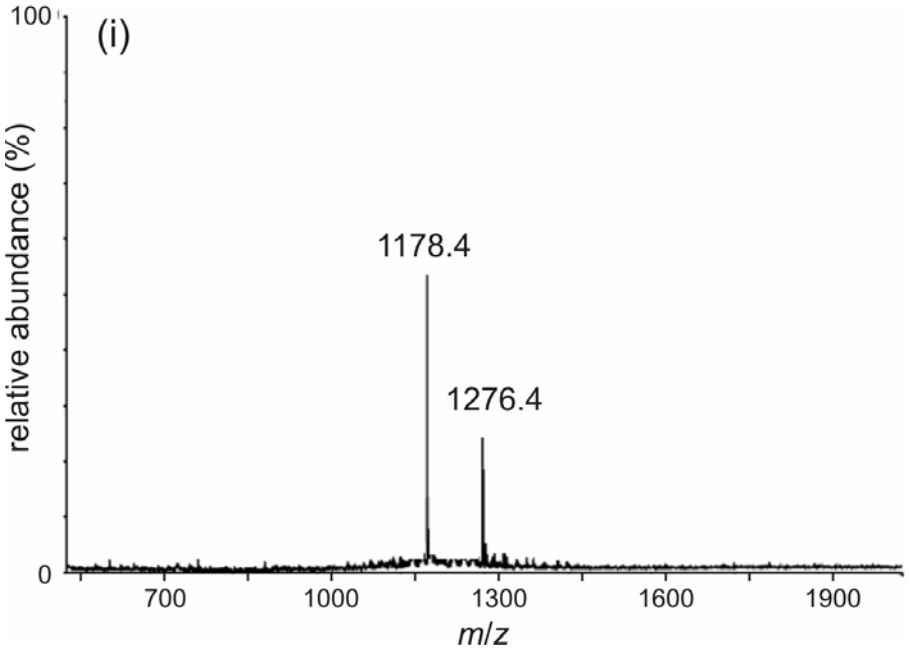
*for*



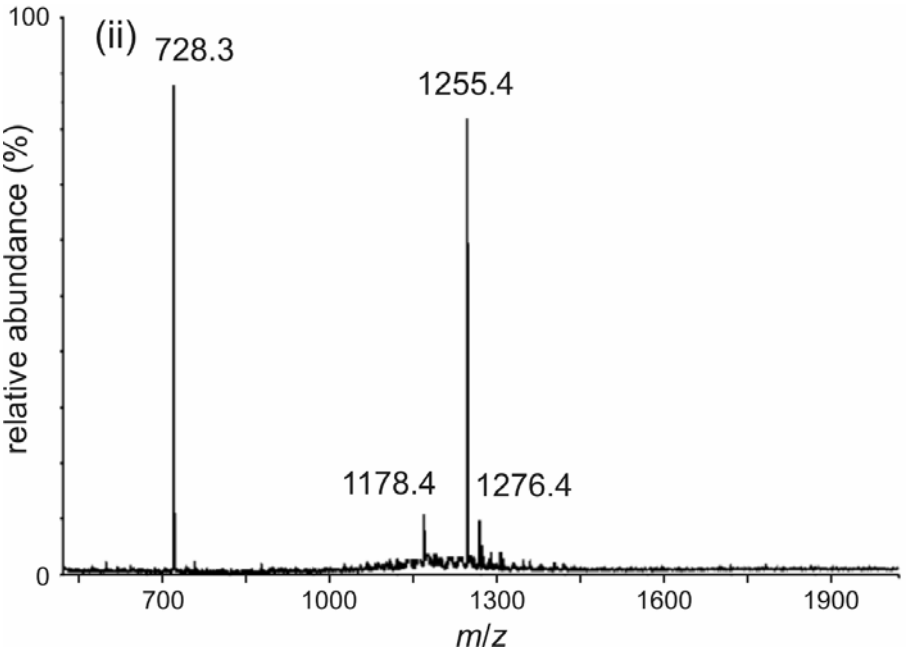

*fragment*

The positive-ion mode mass spectra below refer to:

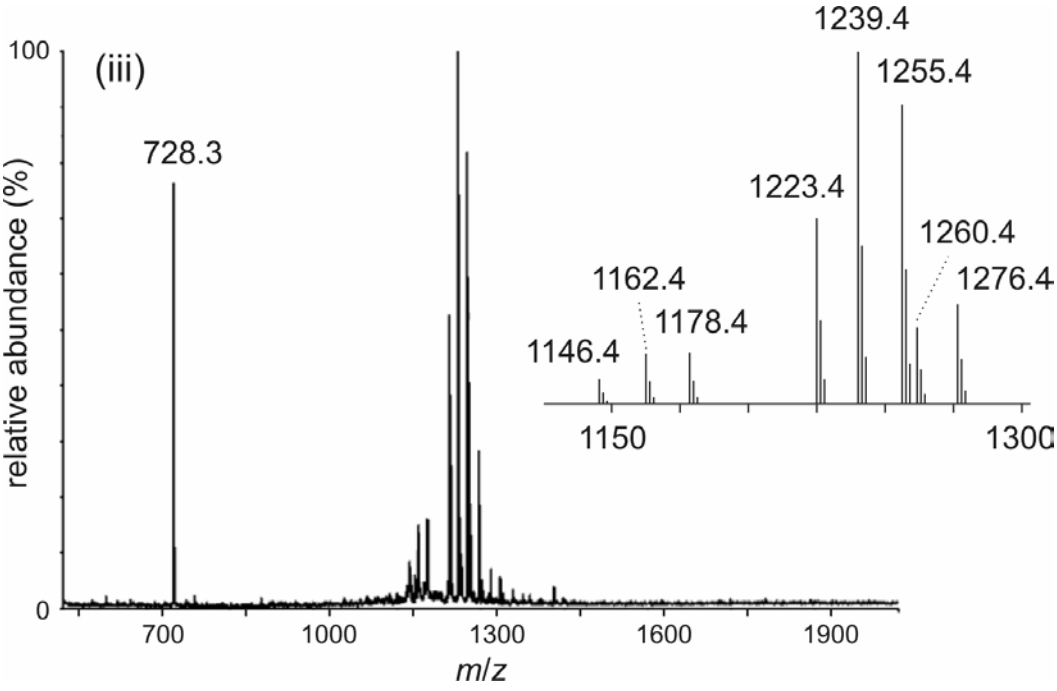
(i) MALDI-MS (IR laser, 2940 nm, dithranol/acid matrix) of the signaling peptide.



(ii) MALDI-MS (IR laser, 2940 nm, dithranol/acid matrix) of the peptide exposed to 5 M KOH, cysteamine and protease.




(iii) MALDI-MS (UV laser, 337 nm, cinnamic acid matrix) of the same sample as in (ii).



a. (10 pts) What parent masses would you expect to see for phosphorylated and non-phosphorylated **YFRPSGF(Y*)D** in positive-ion mode MS? Is either of these masses observed in the spectrum of pure peptide (spectrum *i*)? (*I.e.*, was Shokat's peptide phosphorylated or not?)

| *expected m/z phosphorylated peptide* | *expected m/z, non-phosphorylated peptide* |
|---|---|
| | |

| Was Shokat's peptide phosphorylated? | |
|---|---|

b. (20 pts) When the Shokat group exposed their peptide to base, cysteamine and protease, they observed additional peaks (spectrum *ii*). Identify each of the four labeled peaks in spectrum *ii* by drawing the chemical structure of each corresponding chemical species. You may use any of the one-letter abbreviations given above, but draw out the structure of the *N*-terminus, *C*-terminus, and any modified amino acids in each structure.

| *m/z* = 1276.4 |
|---|
| |

| *m/z* = 1255.4 |
|---|
| |

*m/z* = 1178.4

*m/z* = 728.3

c. (16 pts) There are multiple mechanistic explanations for the peak at *m*/*z* = 1178.4 in spectra *i* and *ii*. Show two sources or fragmentation mechanisms that could generate a *m*/*z* = 1178.4 cation, and describe where in the experiment (*e.g.*, sample prep, laser irradiation, gas-phase fragmentation, etc.) these ions might be produced.

*Explanation #1:*

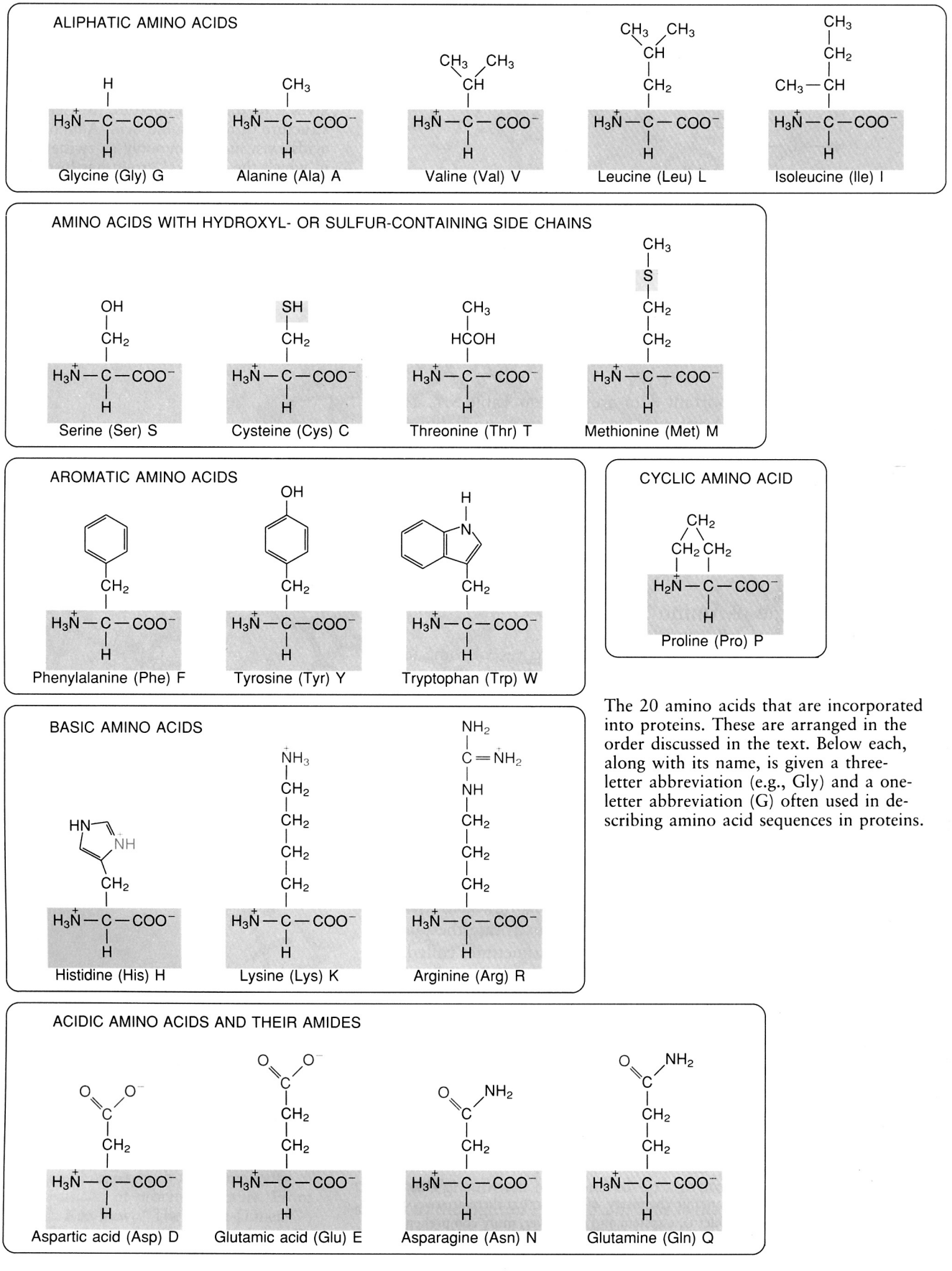
*Explanation #2:*

d. (4 pts) Interestingly, UV irradiation of the peptide MALDI sample produced more fragmentation products than did IR irradiation, even though the total laser power was the same in each case (spectrum *iii*). Shokat's group attributed this to direct absorption of the UV laser light by the peptides in the mixture (rather than just by the MALDI matrix). Which amino acid and/or functional group was responsible for absorbing the 337 nm laser light?

*UV-absorbing amino acid and/or functional group:*

e. (10 pts) All of the additional fragment peaks in spectrum *iii* can be explained by just two fragmentation processes. Draw generic product substructures that describe the products of these two fragmentations, and give the amount of mass lost from the parent in each case. *Do not worry about the fragmentation mechanism here.*

| *fragment substructure 1* | *fragment substructure 2* |
| --- | --- |
| *mass lost from parent:* | *mass lost from parent:* |




The 20 amino acids that are incorporated into proteins. These are arranged in the order discussed in the text. Below each, along with its name, is given a three-letter abbreviation (e.g., Gly) and a one-letter abbreviation (G) often used in describing amino acid sequences in proteins.