

University of Canterbury

End of Year Examination 2008

Prescription Number(s):	CHEM 412 BCHM 412
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Paper Title:	Structural Chemistry and Biology
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Time Allowed: TWO HOURS

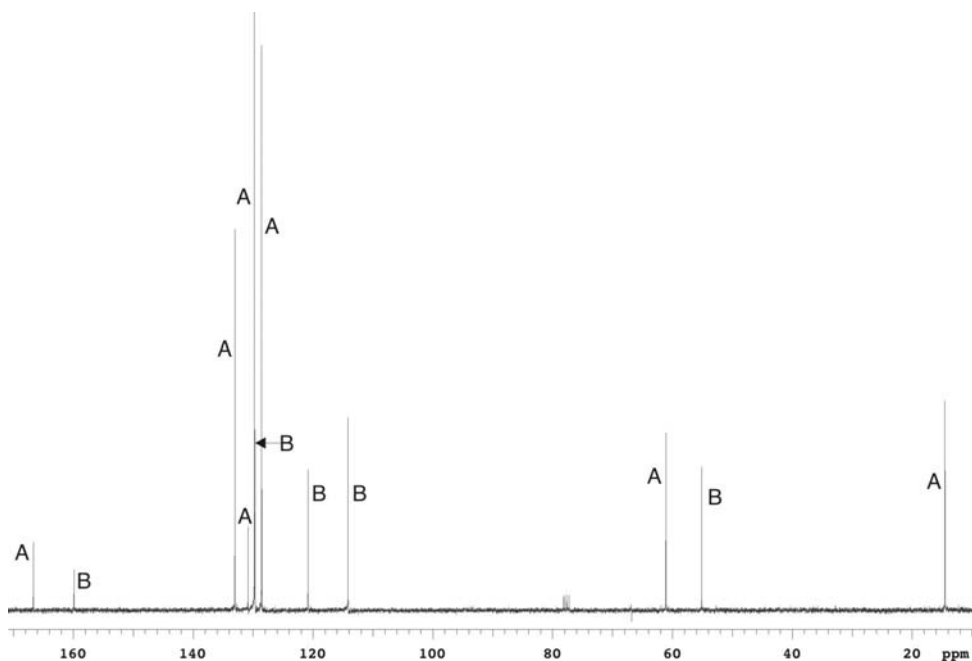
Number of pages: FIVE

Answer **THREE** questions out of **FOUR**.

All questions are of equal value.

TURN OVER

1. Shown below is the ^{13}C NMR spectrum of a mixture of ethyl benzoate (A) and methoxybenzene (B), for which it is required to determine the relative amounts of the two compounds. The spectrum shown has been acquired using the repetitive-pulse FT method with an acquisition time (at) of 1.3 s, an interpulse delay (d1) of 1 s, an excitation pulse of 60° , a sample temperature of 296 K, and with broadband decoupling of protons switched on for the duration of the whole experiment.

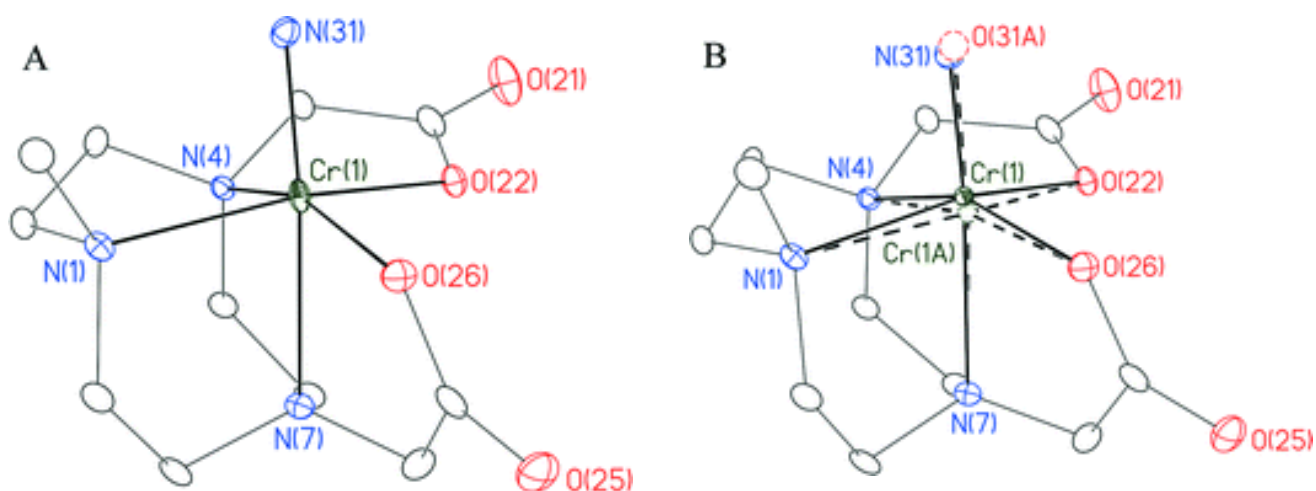


- (a) How could these experimental parameters, or any others, be changed to ensure that, in the resultant spectrum, there will be a 1:1 correspondence between the peak intensity and the number of carbon atoms causing each resonance peak, thereby allowing quantification of the relative amounts of A and B?

Explain how each of the changes you describe would contribute to achieving this 1:1 correspondence.

- (b) Before any parameters are changed, it is necessary to know the relaxation times (T_1) for the carbon atoms causing each resonance peak. Describe the NMR experiment (and its pulse sequence) that would be used to obtain the relaxation times.

2. (a) Single crystal X-ray structure determinations are often considered to be a “gold standard” for identification of a compound. Briefly discuss the reliability of X-ray diffraction techniques for the identification of compounds.
- (b) A recent paper (*Dalton Trans.*, 2008, 1864-1871) describes a crystal structure determination that appeared to reveal a nitridochromium complex with an unusually long chromium-nitrogen triple bond {Cr(1)-N(31) in Figure A}. The origin of this observation was subsequently revealed to be the co-crystallisation of the nitridochromium complex {containing Cr(1) and N(31)} with the hydroxidochromium complex {containing Cr(1A) and O(31A)} that results from partial hydrolysis of the nitride complex during crystallisation. The superimposed structures that were eventually refined are shown in Figure B.
- (i) Is the co-crystallisation surprising? Explain.
- (ii) The shape of the chromium thermal ellipsoid in Figure A is an indication that the refined structure may not be telling the whole story. Explain the purpose of thermal ellipsoids, why this thermal ellipsoid appears the way it does, and why it can be regarded as an indicator of something suspicious. Extend your answer to identify and account for the presence of other (possibly) misleading structural features in Figure A.
- (iii) What other experimental evidence might you look for to support the structural assignment shown in Figure B?



3. **EITHER**

Observation of the nuclear Overhauser effect (nOe) is an important technique in NMR spectroscopy. Describe the origin of this effect, how it can be measured, and discuss a range of situations in which such measurements can aid in the determination of structural properties.

OR

Selective excitation or suppression techniques in ^1H NMR spectroscopy have found many applications in simplifying or enhancing the information that can be obtained from a variety of ^1H NMR experiments. Briefly describe how selective excitation and suppression can be achieved. Give some examples of ^1H NMR experiments that use these techniques, showing the benefits of their use.

4. The following sentence was taken from the abstract of a recent paper (*Biochemistry*, 2008, 47, 4377-4385) that discusses a sulfur transfer enzyme (rhodanese) and a single domain analogue (PspE) that has also been isolated:

“To understand the catalytic mechanism of rhodanese at the molecular level, we determined the solution structures of the sulfur-free and persulfide-intermediate forms of PspE by nuclear magnetic resonance (NMR) spectroscopy and identified the active site by NMR titration experiments.”

- (a) Briefly outline the general steps that are required when determining the structure of a protein by NMR methods.
- (b) How does the size of a protein affect the choice of methods for NMR structure determination? Explain how the methods differ.
- (c) Would the fact that two forms of the enzyme were studied complicate or simplify the problem? Explain.
- (d) Speculate on the nature of the “NMR titration experiments” that might have been undertaken and explain how they might lead to identification of the active site.

END OF PAPER