Experiment 3: Nuclear Magnetic Resonance Spectroscopy – Beyond $^1$H and $^{13}$C

SUGGESTED PRELAB READING:
Skoog Sections: 19A-1
19B-3 (this discussion of splitting is true for all $I=1/2$ nuclei, not just $^1$H!)
19C, (get a general idea of instrument components)
19F/F-1 (with an AWFUL and INCORRECT structure of ATP!)

GOALS:
1. to become familiar with the flexibility and limits of our NMR probe to observe signals from magnetic nuclei other than $^1$H and $^{13}$C
2. to gain experience loading appropriate instrument parameters and tuning the X-channel of our probe to a heteronucleus other than $^{13}$C
3. to collect and interpret $^{31}$P NMR data on several different samples so as to generate some basic trends for phosphorous signal chemical shifts and splitting patterns

TO DO BEFORE OR DURING LAB (PRIOR TO DATA COLLECTION):
Consider/calculate the following:
• At Colby we have a “400 MHz” NMR spectrometer. To which component of the instrument does this designation refer? To which component of the instrument does this designation refer? From the information in this designation, calculate the strength of the magnetic field ($B_0$) of Colby’s instrument in Tesla units.
• The gyromagnetic ratio of the $^{31}$P nucleus is given in Table 19-1 (Skoog) and in the online NMR Periodic Table: http://bouman.chem.georgetown.edu/NMRpt/NMRPerTab.html. Use this value, along with information from the preceding question, to calculate the Larmor precession frequency of a $^{31}$P nucleus on our instrument.
• What is the spin of a $^{31}$P nucleus? Are there any other magnetic isotopes of phosphorous?
• Is $^{31}$P a good NMR nucleus compared to $^{13}$C? Compared to $^1$H?
• What are the CORRECT structures of the following biological compounds (I recommend that you look either in a Biochemistry text book or on a reputable web site):
  o Adenosine 5’-triphosphate (5’-ATP)
  o Adenosine 5’-diphosphate (5’-ADP)
  o Adenosine 5’-monophosphate (5’-AMP)
  o Adenosine 3’,5’-cyclic monophosphate (3’,5’-cAMP)
  o NAD

THE SAMPLES:
For this laboratory experiment you will work independently. At the beginning of lab you will prepare 700 µL of a ~30-60 mM solution of one of the following compounds in D$_2$O. (A not-so-simple question: Why are we using D$_2$O as the solvent?).
  o 5’-ATP
  o 5’-ADP
  o 5’-AMP
  o 3’-5’ cAMP
  o NAD

DATA COLLECTION:
Under the guidance of your laboratory instructor, insert your sample into the NMR spectrometer, and use the ICON NMR program to spin, lock, shim, and collect a quick $^1$H NMR spectrum (4 scans is plenty!) of your sample. Then, following the instructions provided by your lab instructor, load appropriate $^{31}$P parameters and tune the X-channel to phosphorous. Collect a $^{31}$P spectrum of your sample (preferably WITH proton coupling first, and then possibly with $^1$H decoupling if time allows). Print out all spectra. Record all of your file names for your data in your notebook and on the instructor’s lab sheet. Be sure to leave enough time to tune the X-
channel back to $^{13}\text{C}$ before you finish! Please label and photocopy your spectra for your colleagues in lab BEFORE you leave for the day.

**DATA ANALYSIS:**
From the $^{31}\text{P}$ data for each compound, determine for each sample (1) the number of magnetically inequivalent phosphorous nuclei, (2) the number of spin 1/2 nuclei that are close enough to “split” each phosphorous signal, and (3) the possible structure/cooordination/nature of each magnetically inequivalent phosphorous signal. It may be useful to prepare a table of chemical shifts, multiplicities, and coupling constants for comparison among samples. Use these data to identify each sample as one of the unknowns in the list above.

**TO HAND IN FOR YOUR LAB REPORT:**
In a one-page summary, use the class data and your knowledge of NMR spectra to justify your identifications of each sample. Tabular data is appreciated (and appropriate!) although one or two selected figures to illustrate key points may be added beyond the 1-page limit.