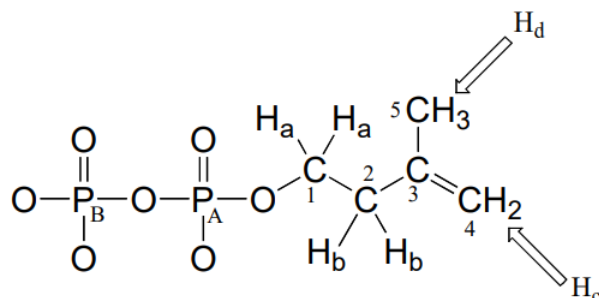


9.10: NMR of phosphorylated compounds

Because so many biological molecules contain phosphoryl groups, it is worthwhile to look at how scientists use NMR to determine the structure of these molecules. Recall from section 5.1 that ^{31}P , the most abundant isotope of phosphorus, is *NMR* active: it can be directly observed by ^{31}P -*NMR*, and indirectly observed in ^1H -*NMR* and ^{13}C -*NMR* through its spin-coupling interactions with neighboring protons and carbons, respectively.

Consider the case of isopentenyl diphosphate, the building block molecule used by cells to make 'isoprenoid' compounds such as cholesterol (in many animals), or β -carotene (in some plants). *NMR* spectra of this molecule were taken in a D_2O solvent, buffered with ND_4OD (the deuterium equivalent of aqueous ammonium hydroxide, NH_4OH) (J. Org. Chem. 1986, 51, 4768). In our discussion, carbon atoms are specified with numbers, protons with lower case letters, and phosphorus atoms with upper case letters.



First, let's look at the proton spectrum:

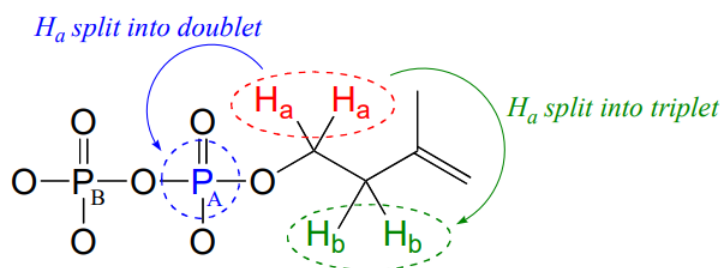
^1H -*NMR*

H_a : 4.05ppm(t_d), $^3J_{H_a-H_b} = 6.6$ Hz; $^3J_{H_a-P_A} = 3.3$ Hz.

H_b : 2.39ppm(t), $^3J_{H_a-H_b} = 6.6$ Hz

H_c : 4.86ppm(s)

H_d : 1.77ppm(s)



The signals for H_b , H_c , and H_d look like we would expect from our discussion in chapter 5, with the exception of H_c which you will be invited to discuss in the exercise below. Why, though, is the signal for H_a split into a triplet of doublets (td)? First of all, as expected, the two neighboring H_b protons split the H_a signal into a triplet, with $3J_{\text{H-H}} = 6.6$ Hz. Then, **the signal is further split into doublets** ($^3J_{\text{H-P}} = 3.3$ Hz) by P_A , **the closer of the two phosphorus atoms**. A phosphorus atom will

? Exercise 9.10.1

The signal for the two ' H_c ' protons in isopentenyl diphosphate is reported above as a singlet integrating to $2H$. Are these two protons really chemically equivalent, and, according to what you know about proton *NMR*, should this signal really be a singlet? If not, what kind of signal(s) would you expect to see? Explain any discrepancies between what you would expect to see and the actual reported data.

Now, let's look at the ^{13}C spectrum of IPP:

$^{13}\text{C} - \text{NMR}$ (proton-decoupled)

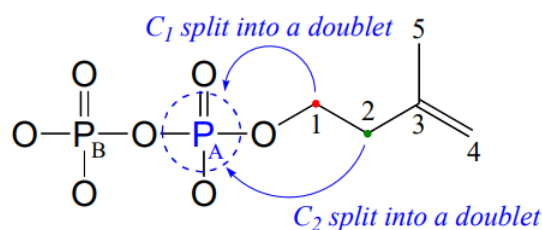
C_1 : 40.7ppm(d);² $J_{C_1-P_A} = 7.2$ Hz

C_2 : 67.0ppm(d);³ $J_{C_2-P_A} = 4.0$ Hz

C_3 : 147.4ppm

C_4 : 114.6ppm

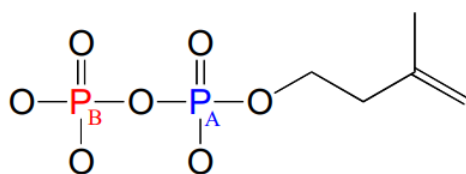
C_5 : 24.5ppm



Notice that the signals for both C_1 and C_2 are split into doublets by the magnetic field of P_A . Phosphorus atoms will spin-couple with ^{13}C nuclei up to three bonds away. Notice also that the 2-bond coupling between C_1 and P_A is larger than the 3-bond coupling between C_2 and P_A (7.2 Hz vs. 4.0 Hz). Finally, notice that we do not observe 4-bond $C - P$ coupling: C_3 is not spin-coupled to P_A , and P_B is not coupled to any of the ^{13}C or ^1H nuclei on the molecule.

Remember that when processing a typical $^{13}\text{C} - \text{NMR}$ spectrum, we electronically 'turn off' spin coupling between carbons and neighboring protons in order to simplify the spectrum (this is referred to as 'proton decoupling'). Proton decoupling does not turn off $C - P$ spin coupling.

Because ^{31}P is NMR -active, we can also, with an NMR spectrophotometer equipped with a phosphorus probe, directly observe the phosphorus NMR signals, just as we can directly observe the signals from protons and ^{13}C nuclei. On an NMR instrument where protons resonate at 300 MHz and ^{13}C nuclei resonate at 75 MHz, phosphorus resonates at 32 MHz. In $^{31}\text{P} - \text{NMR}$ experiments, the reference standard used to determine the 0 ppm point is usually phosphoric acid (tetramethylsilane, the standard 0 ppm point for ^1H - and $^{13}\text{C} - \text{NMR}$, doesn't have a phosphorus atom!). The $^{31}\text{P} - \text{NMR}$ spectrum of isopentenyl diphosphate has, as expected, two peaks, each of which is upfield of the phosphoric acid standard (negative chemical shifts!) and split into a doublet ($^2J_{P-P} = 20$ Hz) due to 2-bond coupling between the two phosphorus nuclei.



P_A : (-)11.03 ppm (d, $^2J_{P-P} = 20$ Hz)

P_B : (-)7.23 ppm (d, $^2J_{P-P} = 20$ Hz)

Notice that although the C_1 and C_2 signals were split by P_A in our $^{13}\text{C} - \text{NMR}$ spectrum, in the $^{31}\text{P} - \text{NMR}$ spectrum the converse is not true: the P_A signal is not split by C_1 or C_2 . Both of these carbons are NMR -inactive ^{12}C isotope in 99 out of 100 molecules. In addition, $P - H$ splitting is not observed in this ^{31}P spectrum, because proton decoupling is in effect.

This page titled [9.10: NMR of phosphorylated compounds](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Tim Soderberg](#) via [source content](#) that was edited to the style and standards of the LibreTexts platform.