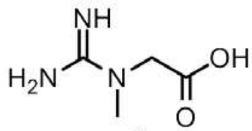
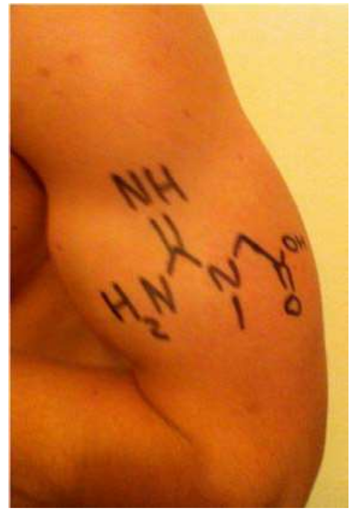
**5. This question is about getting big muscles**

Creatine has recently become one of the most widely used nutritional supplements among athletes. Although there is much debate about which of the advertised beneficial effects of creatine are actually true, the use of creatine is generally believed to lead to a short-term gain in body mass/muscle size. The structure of creatine is shown below.




creatine

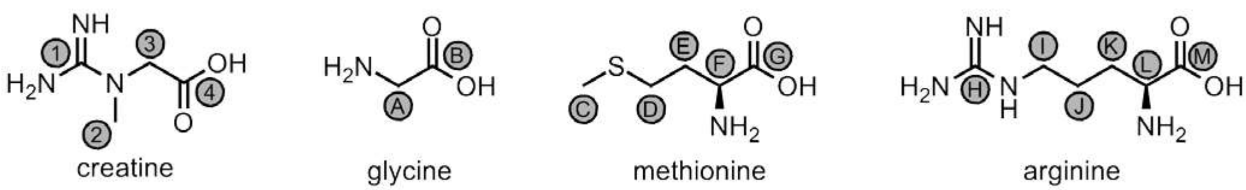



In the body, creatine is converted into phosphocreatine which is used as an energy reserve in the muscles that can be rapidly mobilised to convert adenosine diphosphate (ADP) back into adenosine triphosphate (ATP) – the body's energy currency – in times of need.

**(a)** Creatine is often sold in capsules labelled as 'Pure Creatine Monohydrate'. Write the molecular formula of creatine monohydrate.

**(b)** Creatine is synthesised naturally in organisms from three amino acids: glycine, methionine and arginine.

In your answer booklet, for each of the carbon atoms in creatine (labelled as **1-4**), suggest which carbon atom of the three amino acids it came from (labelled as **A-M**)




**(c)** Like amino acids, creatine exists in different ionised forms depending on the pH of the solution it is in. This causes the overall charge on the molecule to vary. Draw the most common form of creatine at each of the following pHs (the overall charge on the molecule at each pH is given).

**(i)** pH 1 (overall charge = + 1)

**(ii)** pH 7 (overall charge = neutral)

**(iii)** pH 12 (overall charge = – 1)

The chemical structure of creatine and these amino acids can be analysed by $^{1}H$ NMR.

As these are polar molecules, the NMR spectra are run in $D_2O$ solvent. In $D_2O$, protons attached to nitrogen or oxygen atoms undergo rapid exchange with deuteriums from the solvent. This means that by the time the NMR is run, all N-H bonds have been replaced by N-D bonds and all O-H bonds by O-D bonds. As signals from deuterium atoms are not observed in $^{1}H$ NMR spectra, no signals from N-H or O-H groups in the molecule are seen in the spectrum.

The number of signals observed depends on the symmetry of the molecule. Each hydrogen atom in a unique environment gives rise to a signal at a different chemical shift in the spectrum. Occasionally, signals from two different environments can appear on top of one another when the difference in chemical shifts between the environments is very small.

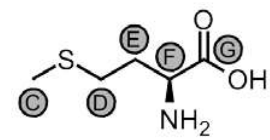
The area under each signal is proportional to the number of protons in that environment. This is shown by an integral trace (the stepped line on the spectrum). The height of each step is proportional to the area under that signal.

The appearance of the signals can be complicated by coupling. If the hydrogen atom(s) are within three bonds of another hydrogen which is in a different environment, instead of appearing as a single peak, its signal is split into a number of peaks. If the hydrogen under consideration is within three bonds of **n** hydrogens in a different environment from itself, it will be split into **(n + 1) equally spaced** peaks. The ratio of the area under the peaks is given by the number in Pascal's triangle (shown on the right). Due to rapid exchange of any protons/deuteriums bonded to oxygen or nitrogen atoms with the solvent, no coupling is seen to protons/deuteriums bonded to oxygen or nitrogen atoms.

| **n** | **Intensities of peaks** |
|---|---|
| 0 | 1 |
| 1 | 1 : 1 |
| 2 | 1 : 2 : 1 |
| 3 | 1 : 3 : 3 : 1 |
| 4 | 1 : 4 : 6 : 4 : 1 |

**(d)** Consider the amino acid methionine.

Complete the table in the answer booklet for carbons **C**, **D** and **F** in methionine to suggest the appearance of the overall signal from the protons bonded to that carbon atom.




methionine

**(e)** Usually all protons attached to the same carbon atom are in the same chemical environment; however, this is not always the case. Two protons on the same carbon atom that are in different chemical environments are called diastereotopic protons.

These are most often observed where the carbon under consideration is bonded to an asymmetric carbon atom. An asymmetric carbon atom has four different chemical groups attached to it.

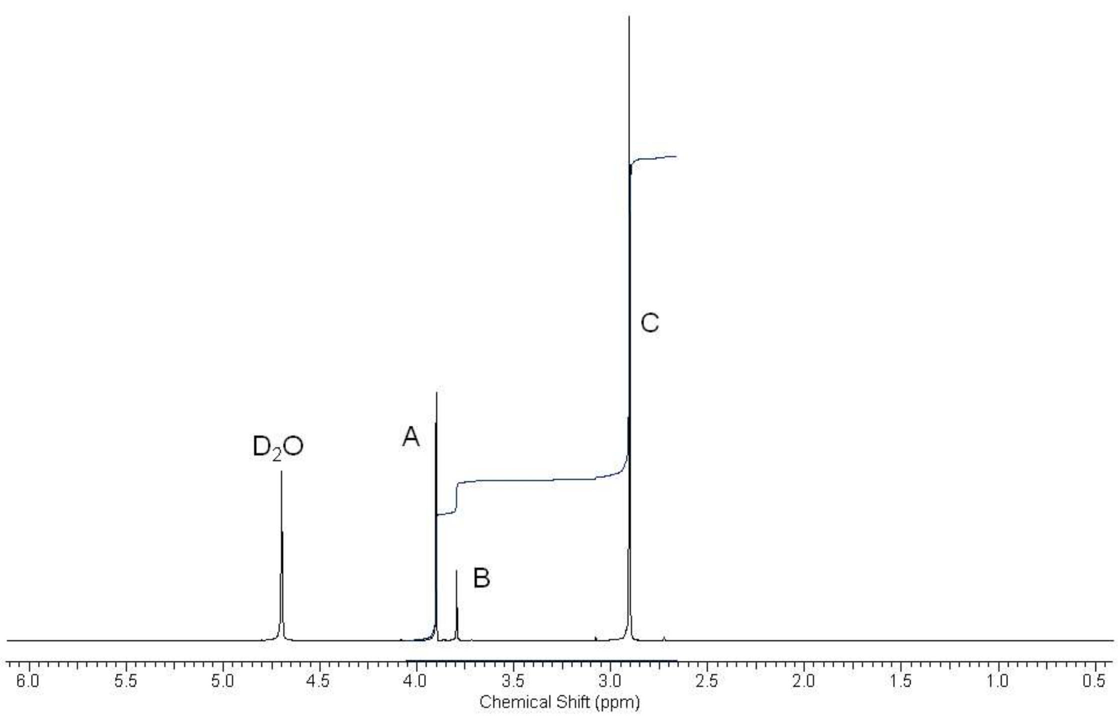
Consider glycine, methionine and arginine. In these three amino acids, write the letters of all such carbons whose diastereotopic protons would be observed as different signals in their spectra.

In the body, creatine is in equilibrium with a cyclic molecule called creatinine, by the following equation. The position of equilibrium varies with pH.

$$\text{creatine} \overset{K}{\rightleftharpoons} \text{creatinine} + H_2O$$

Creatinine is a metabolic waste product that is not used by the body. It is filtered out in the kidneys.

The $^1$H NMR spectrum in $D_2O$ of a creatine/creatinine solution is shown below. Three signals are observed. Creatinine gives rise to signal A. Creatine gives rise to signal B. Both creatine and creatinine give rise to signal C.



**(f)** Suggest a structure for creatinine.

**(g)** Assuming this sample has reached equilibrium, calculate a value for the equilibrium constant, $K$, at this pH and temperature. Show clearly how you worked this out. You may ignore the concentration of water in your calculation.

**(h)** A problem with creatine supplementation is that a lot of the creatine taken does not get absorbed by the body. Recently, supplements containing derivatives of creatine have been marketed. These are usually more lipophilic (dissolve more easily in fats) in an effort to improve uptake into the body.

The $^{1}H$ NMR spectrum in $D_2O$ of one of these supplements is shown below. Some regions of the spectrum have been expanded on the left hand side of the figure to help with your analysis.

This supplement exists in an ionised form at pH 1 but does not exist in an ionised form at pH 12.

Suggest a structure for this supplement.