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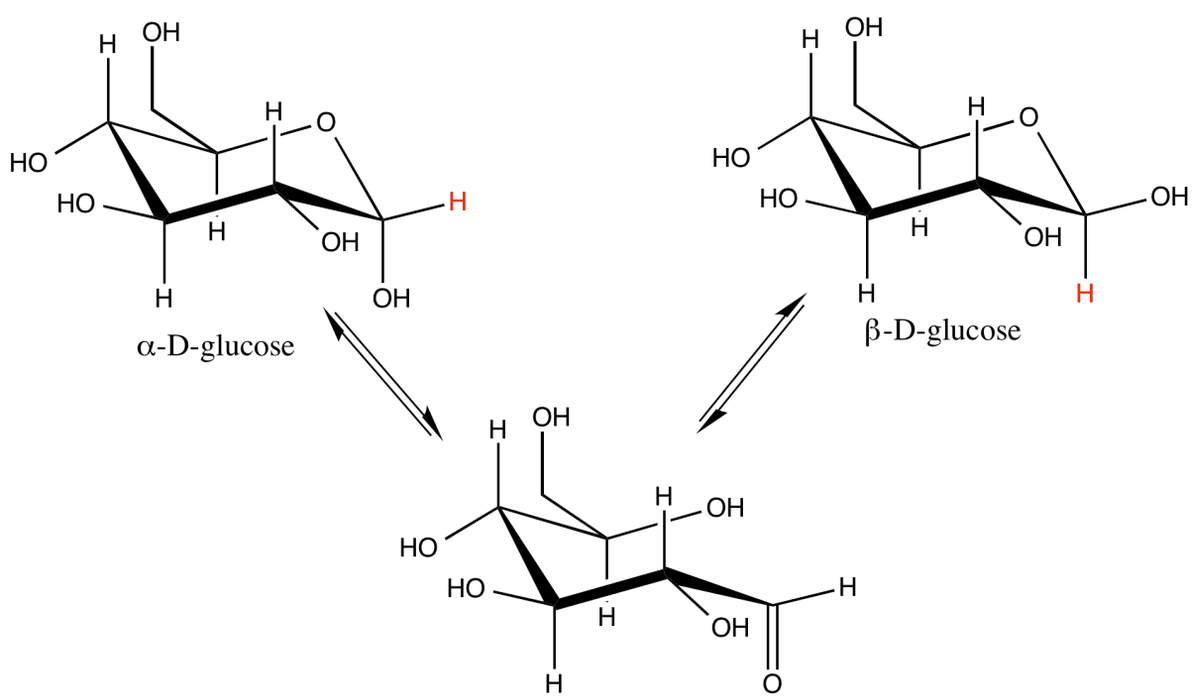
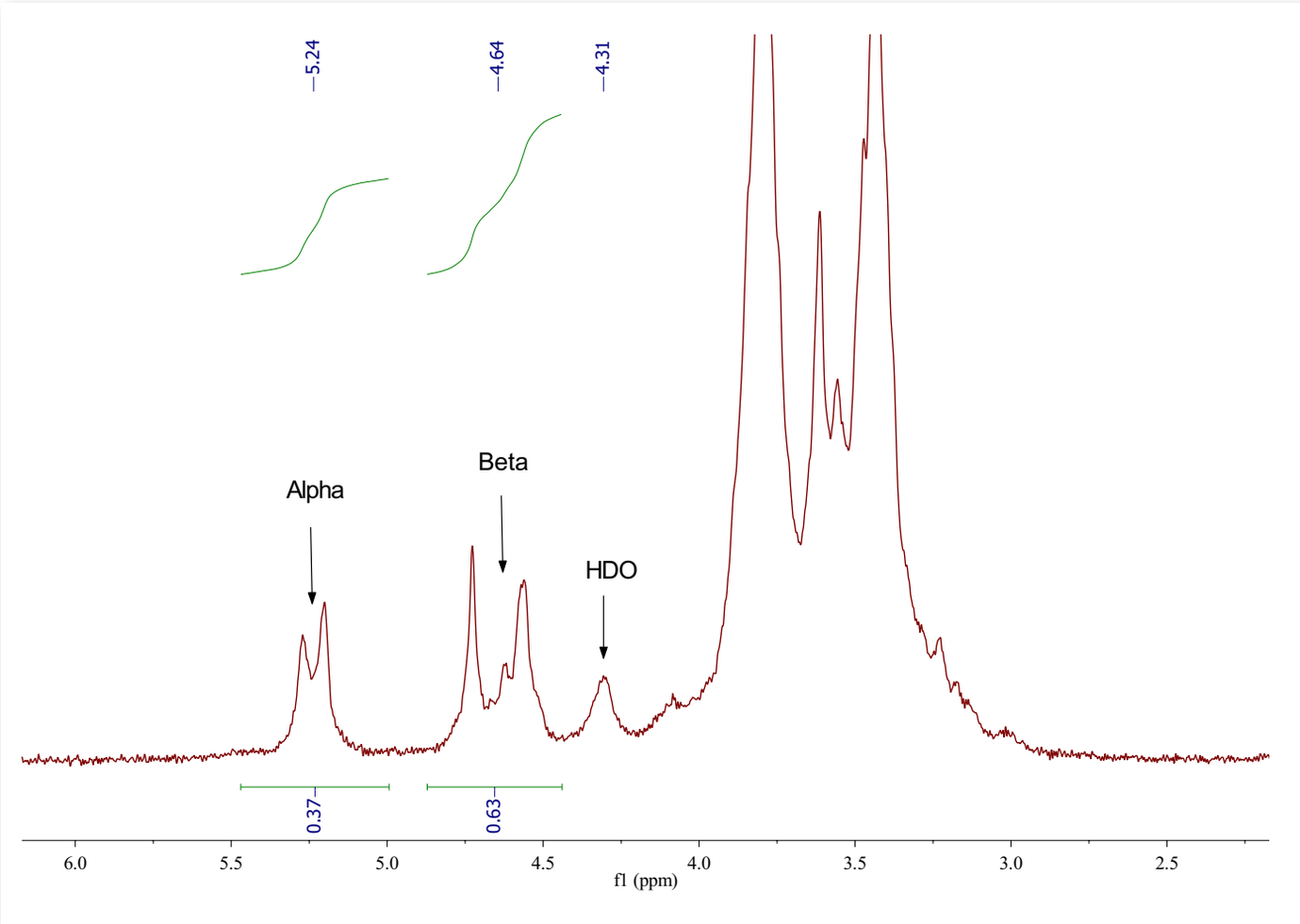
Application Note 5: Glucose Anomers



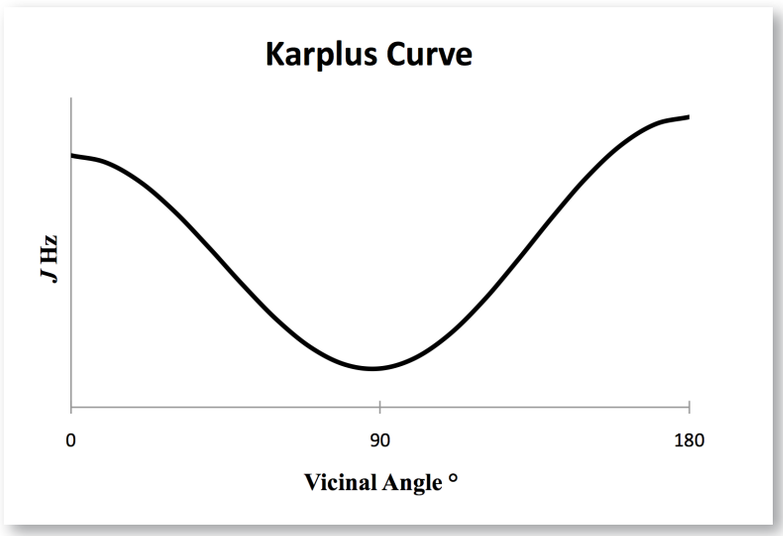
Conformational Analysis by NMR

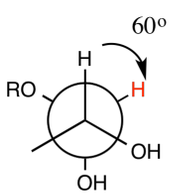
Glucose in solution remains mostly in the cyclic pyranose form in two conformational anomers shown in the figure above. These two forms interconvert via an aldehyde chain. The anomeric rotation can be observed by monitoring the ^{1}H NMR spectrum of the anomeric protons shown in red above. These protons inherit two different characteristics due to their local environment: chemical shift due to shielding from the external magnetic field and *J*-coupling ($J_{H\text{-}H}$) from protons on the adjacent carbon (C2). The additional shielding of the oxygen shifts the anomeric protons down field from the rest of the non-exchanging protons allowing us to resolve them from the others, which occur between 3 and 4 ppm. In addition the equatorial (*alpha*) anomeric proton resonates further down field (5.1 ppm) from the axial (*beta*) proton (4.5 ppm) making these two anomer populations distinguishable by ^{1}H NMR even at low field.

The spectrum below was collected on deuterium exchanged glucose to decrease the HDO signal and heated to 90° C to shift it to 4.3 ppm. The spectrum shows the two anomers clearly resolved. The natural distribution is 36% *alpha* and 64% *beta*, which is represented in the relative integrals of the two NMR peaks.

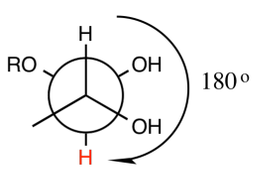


The second distinguishing characteristic is the spin-spin coupling (J_{H-H}). Non-equivalent protons on the adjacent carbon (C2) will couple to the anomeric proton creating a doublet. In rigid molecules where rotational averaging is negligible, this coupling will not average out and will depend on the dihedral angle between the two coupled protons. Martin Karplus presented this relation in the 1960s and would go on to win a Nobel Prize for this work in 2013. In glucose the *alpha* anomer has a dihedral angle of 60 degrees and a coupling constant of about 2.7 Hz, while the *beta* anomer has an angle of 180 degrees and a coupling constant of about 7.2 Hz.

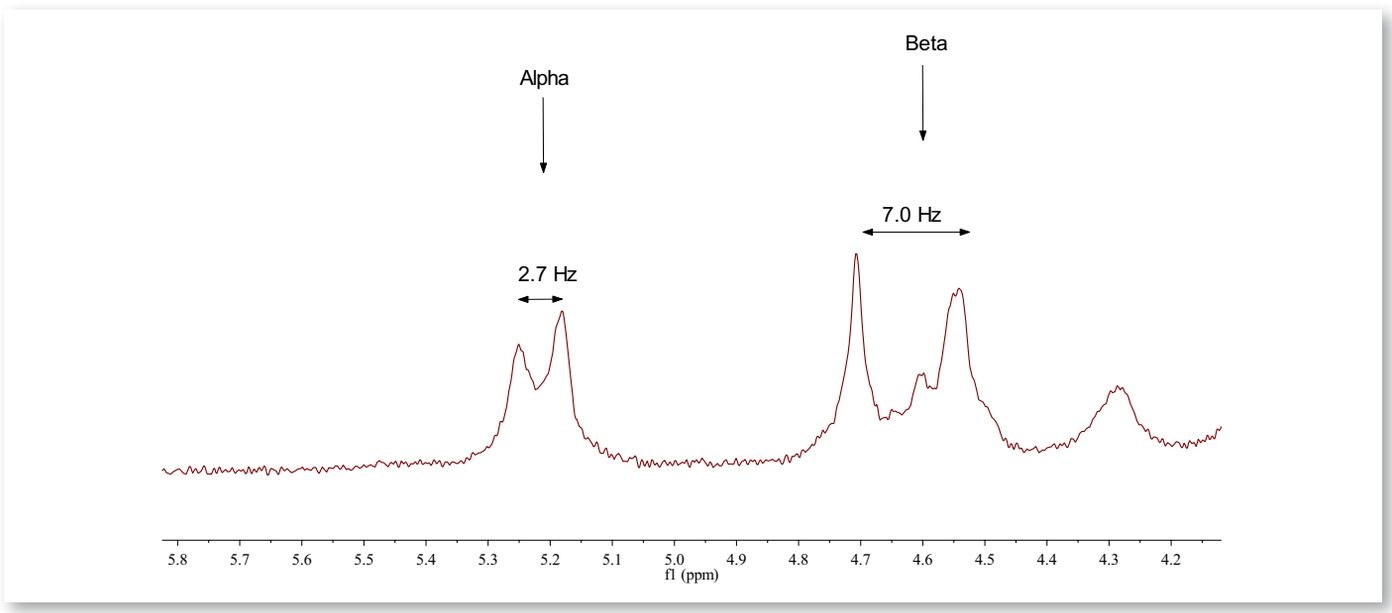




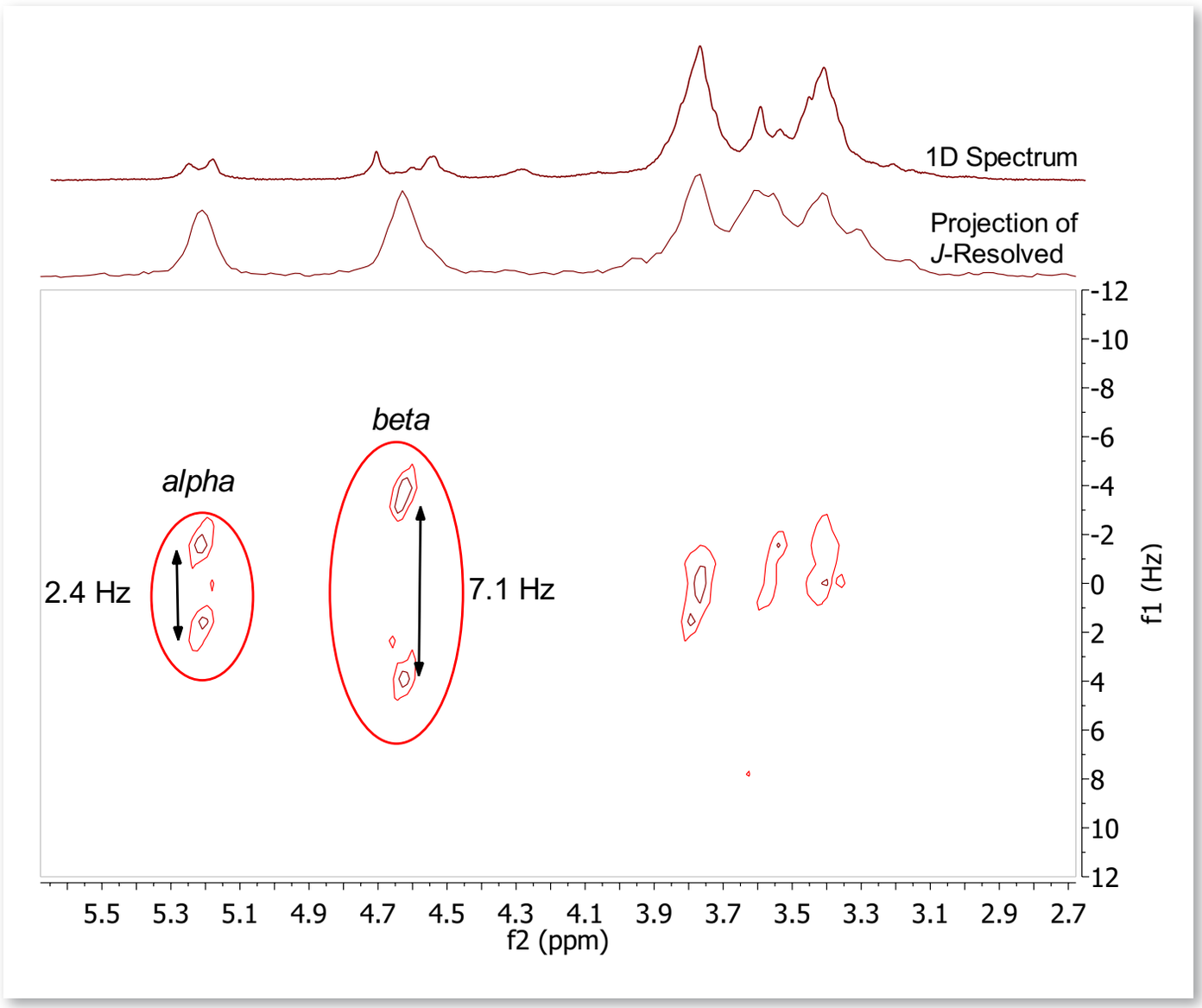
α-D-glucose



β-D-glucose



These couplings are also shown in the *J*-resolved NMR experiment below.



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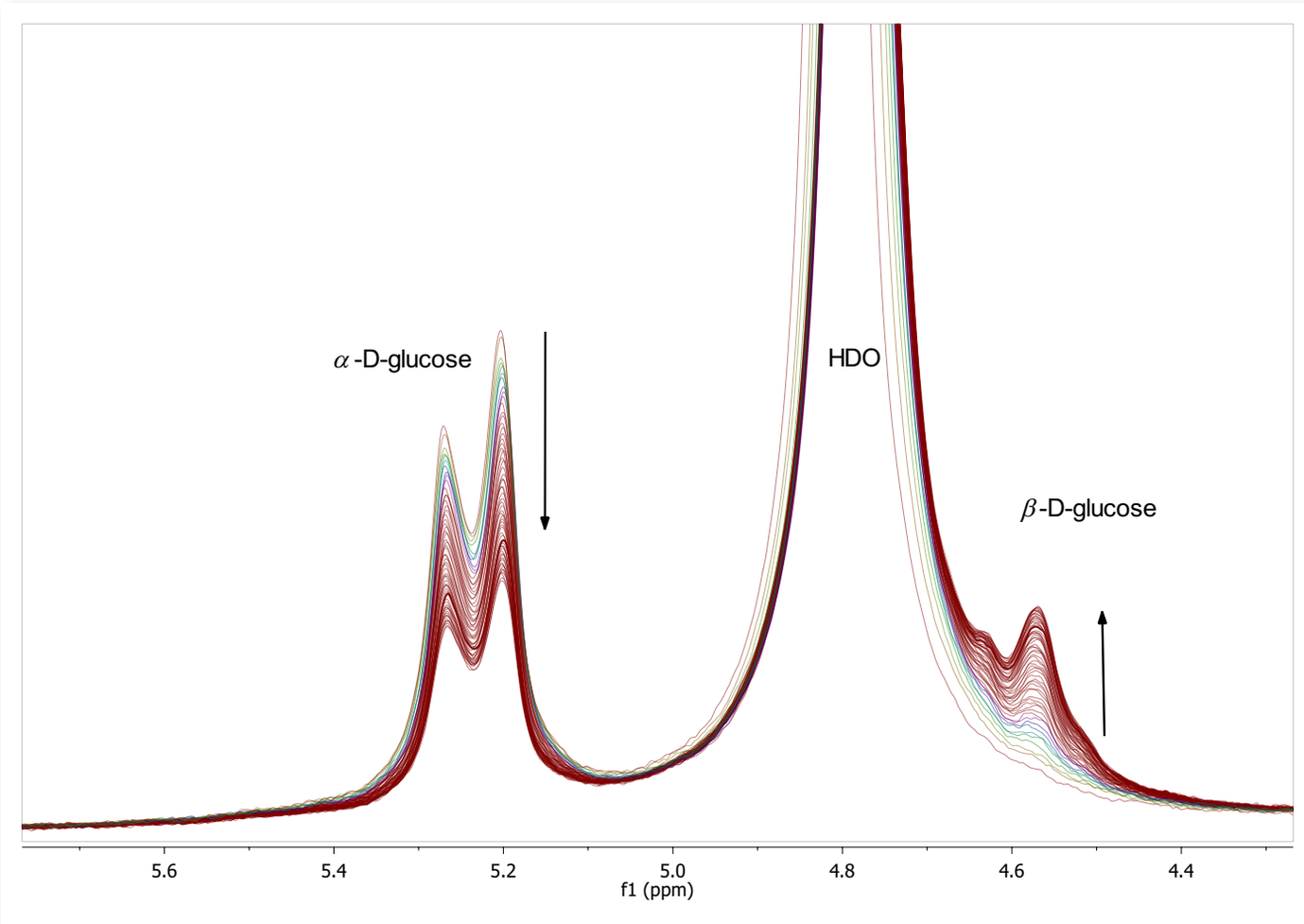
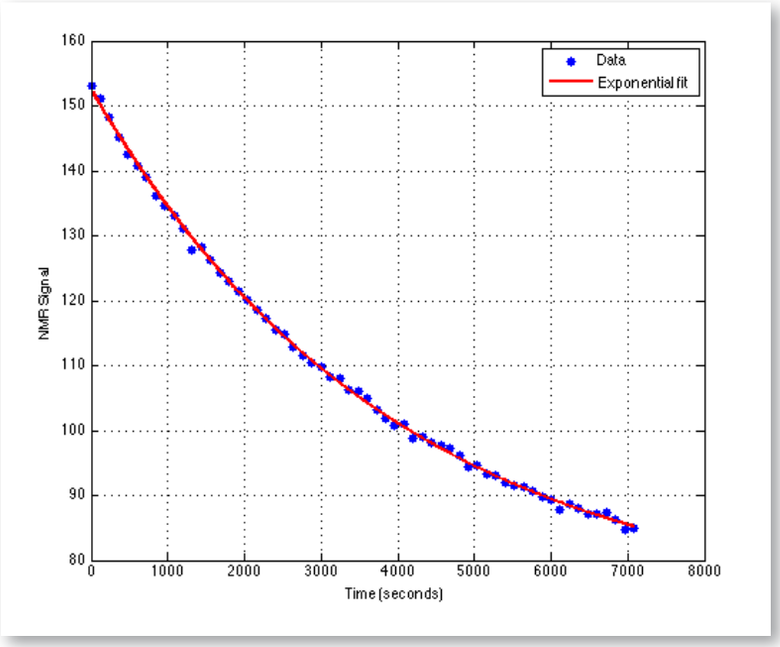
Monitoring Reaction Kinetics by NMR



It is possible to monitor the mutorotation reaction by NMR because the two proton peaks that represent the different anomers are spectrally resolved. About 80 mg of α-D-glucose was dissolved in 0.8 mL D_2O and then immediately transferred to an NMR tube and inserted into the Spinsolve NMR spectrometer. The new reaction monitoring scripting procedure was used to collect a 4 scan average every two minutes for two hours.



The reaction starts instantly and can be observed by watching the concentration of the *alpha* anomer decrease (peak at 5.1 ppm) and the concentration of the *beta* anomer increase (peak at 4.45 ppm). The peak at 5.1 ppm was integrated for each spectrum and plotted against time. The data was fit to an exponential function and a rate of 0.015 min^{-1} was measured at about 26°C, which agrees well with the literature.

Going beyond

- The reverse reaction can also be monitored starting with pure *ß*-D-glucose, but this anomer tends be more difficult to find in a pure state.
- The reaction can be run at other temperatures to determine the activation energy, but the reaction needs to be run outside the magnet and sampled in aliquots.
- The *beta* species can also be monitored by integrating the whole water peak and *beta*-anomeric proton peak. The fit will pull out the water integral as a constant.

Reference:
Drake, E.N. and Brown C.E., *J. Chem Ed*. **54**, 124 (1977).

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