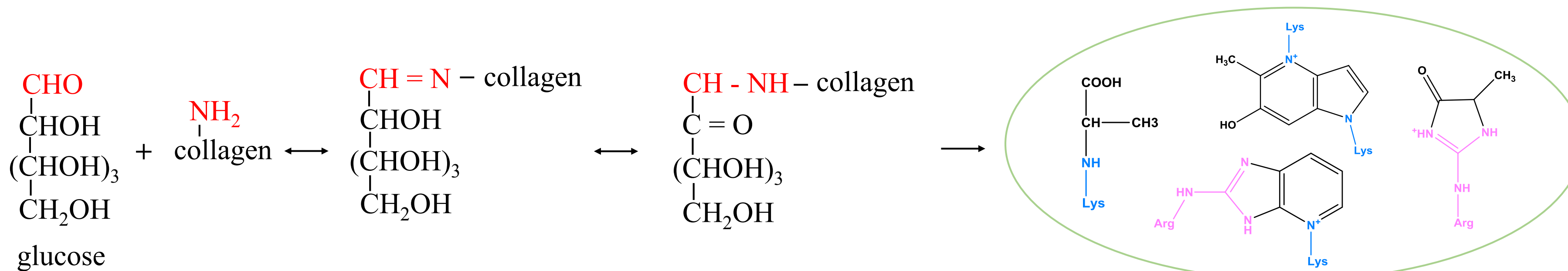


Using solid-state NMR to study the chemistry of diabetes and diabetic complications

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Why diabetes cause complications?

- Diabetes and ageing both lead to similar symptoms, including multiple essential organ failure. Collagen glycation is widely believed to play an important role in this process.
- Extracellular matrix (ECM), basically a collagen scaffold, is a long lived structure with slow turn over rate so it is most likely to be affected by glycation, causing detrimental long term effects.
- Glycation is a non-enzymatic reaction between amino groups in proteins and carbonyl groups in open chain sugar, resulting in amino acid sidechain modifications and intermolecular cross links. The reactions and some glycation products are shown below.
- Glycation is not limited to sugar, as metabolic ketos and aldehydes particularly in hyperglycaemia and oxidative stress, are also reactive glycation agents.



METHODS & OBJECTIVES

- Use mammalian cell culture to produce U-¹³C, ¹⁵N-labelled ECM
- Incubate ECM with glycating agents
- Use multiple analytical methods, including solid-state NMR, TEM and SEM to study the structural effects of glycation reactions on ECM
- Understand the molecular basis underlying ageing and diabetic complications



What happens when proteins are surrounded by sugar?

Glycation Reaction between U-¹³C, ¹⁵N-ECM and ¹³C-Ribose

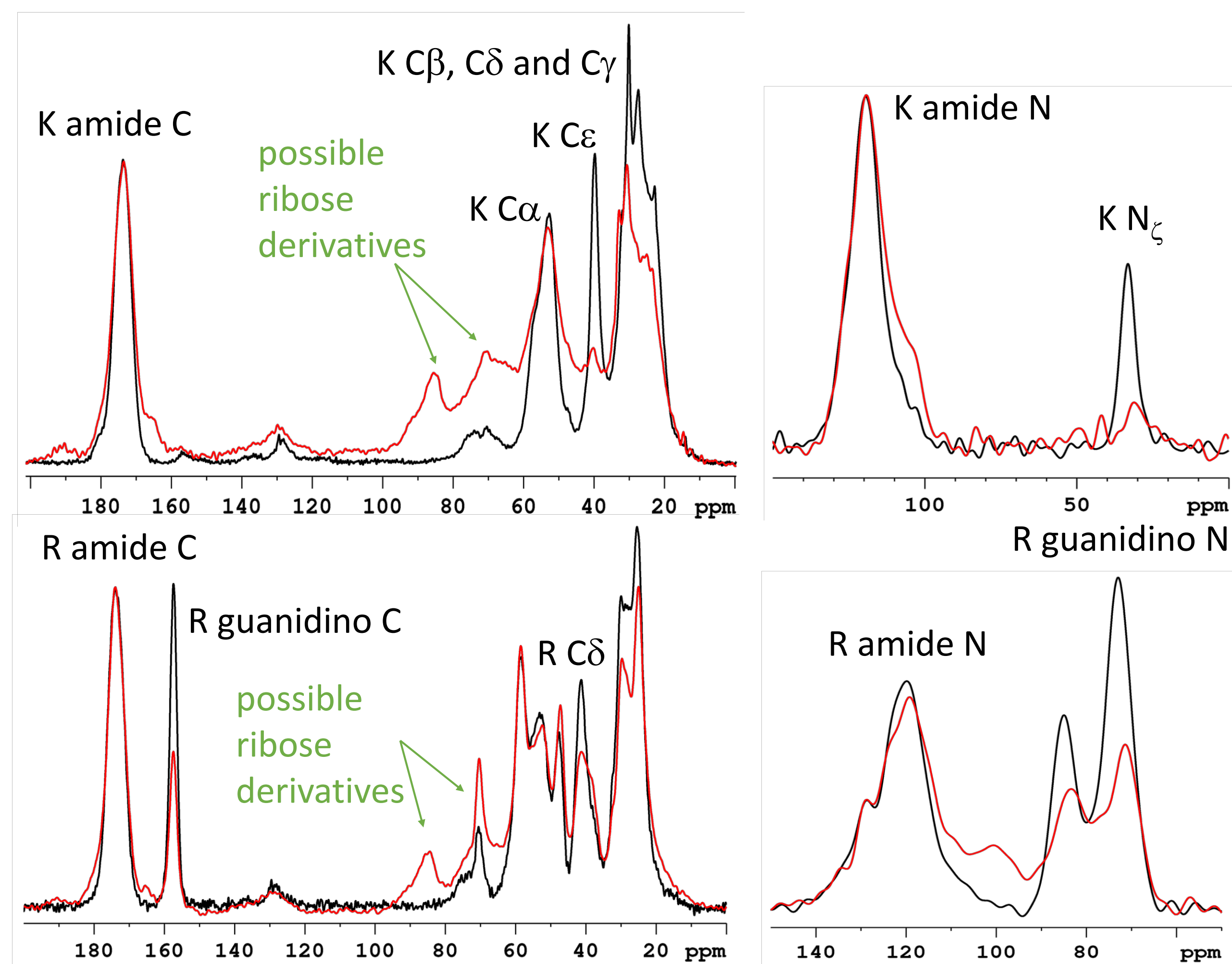


Figure 1. Overlays of the 1D ¹³C (left) and ¹⁵N (right) CP spectra before (black) and after (red) glycation, confirming the reaction between ECM and ribose and that Lys (K) N_ζ and Arg (R) N_η are involved

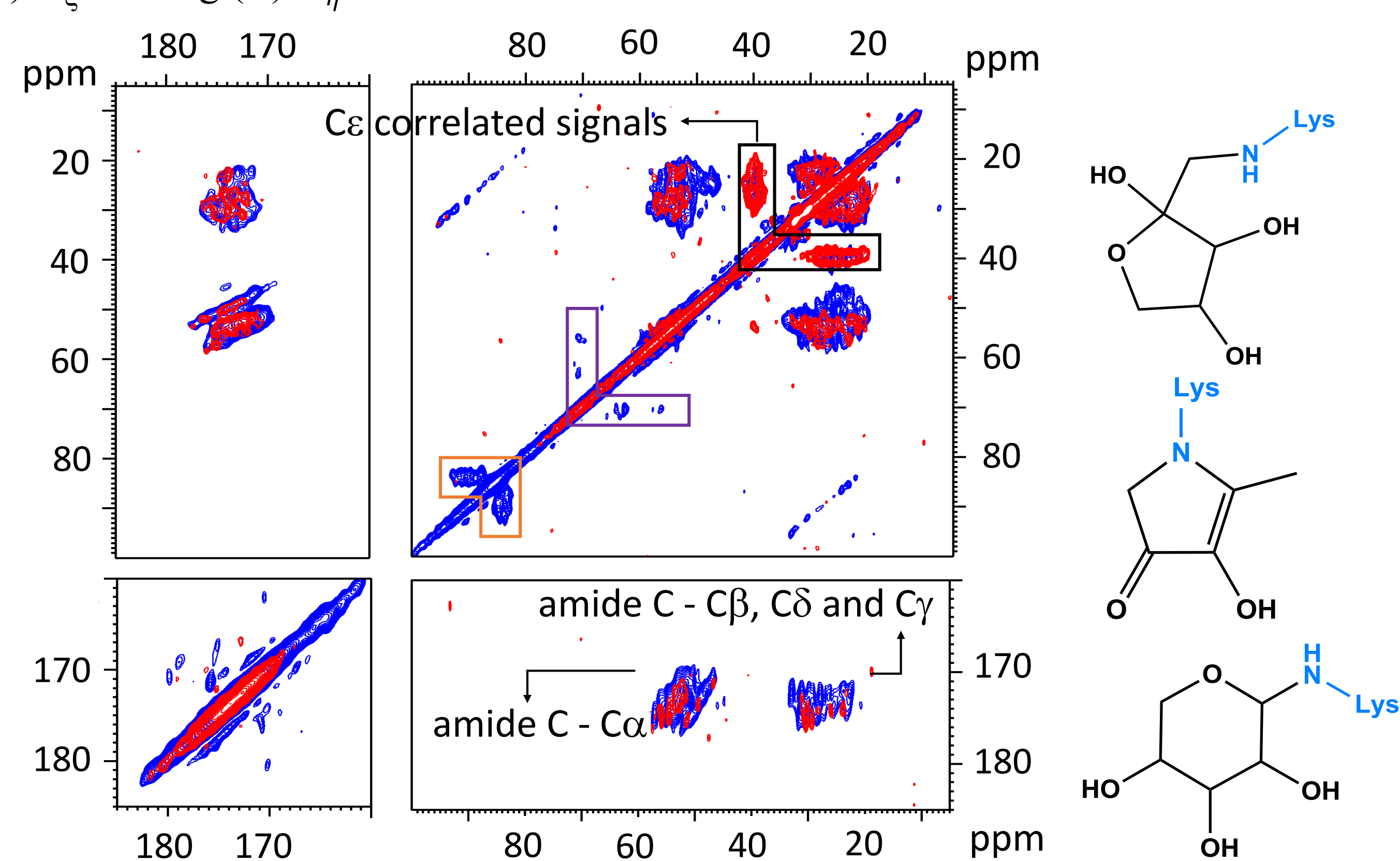


Figure 2. Comparison between 2D ¹³C-¹³C PDS spectra before (red) and after (blue) glycation of ¹³C, ¹⁵N-Lys ECM with ¹³C-ribose, and some possible glycation products with the data

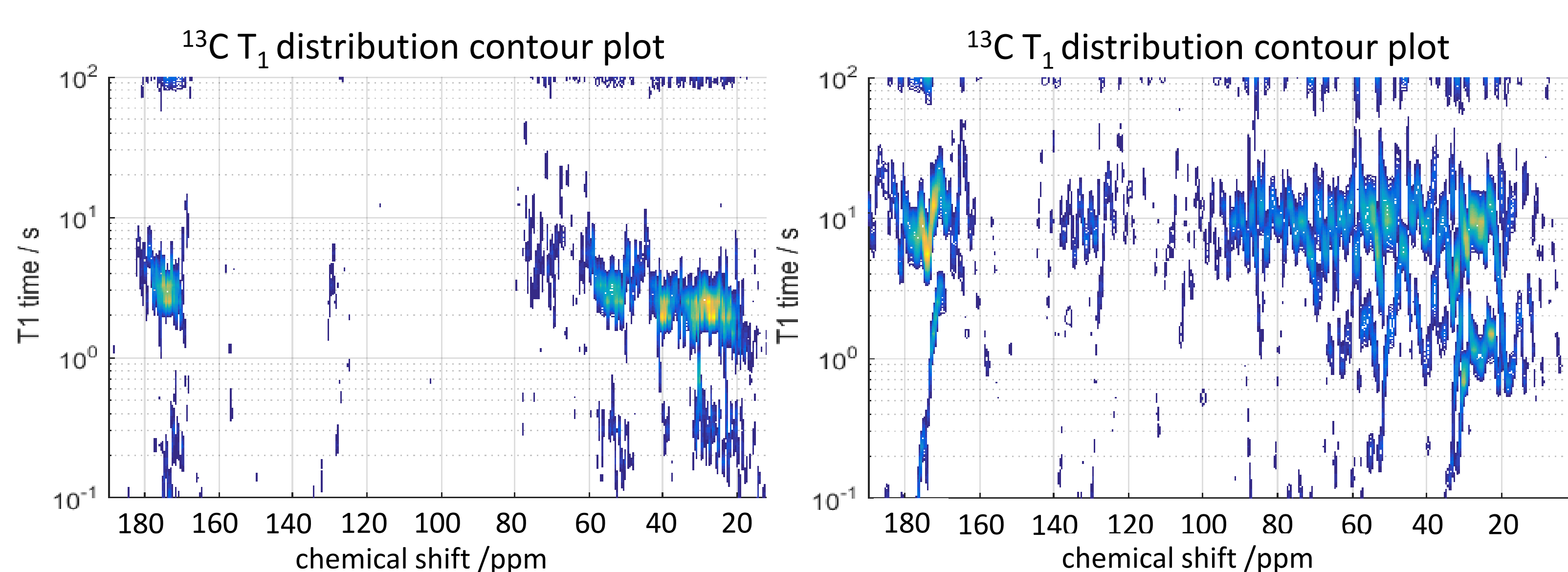


Figure 3. Illustration of the dynamic changes before (left) and after (right) glycation reaction between ¹³C, ¹⁵N-Lys ECM and ¹³C-ribose. Inverse Laplace Transform separates the T₁ relaxation decay of different components and displays these as contour plots.

Glycation Reaction using ¹³C-Ribose-5-Phosphate (R5P)

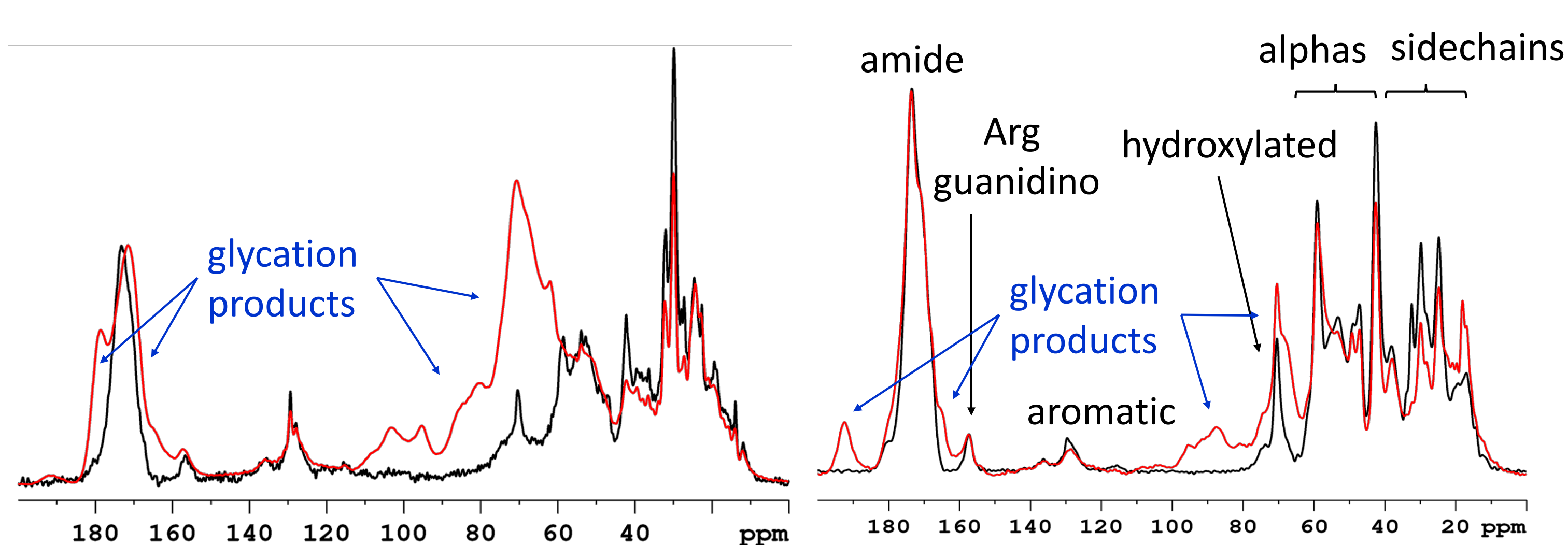


Figure 4. Overlays of the 1D ¹³C CP spectra of ECM (left) and pure collagen (right) before (black) and after (red) glycation reaction, showing a number of similarities

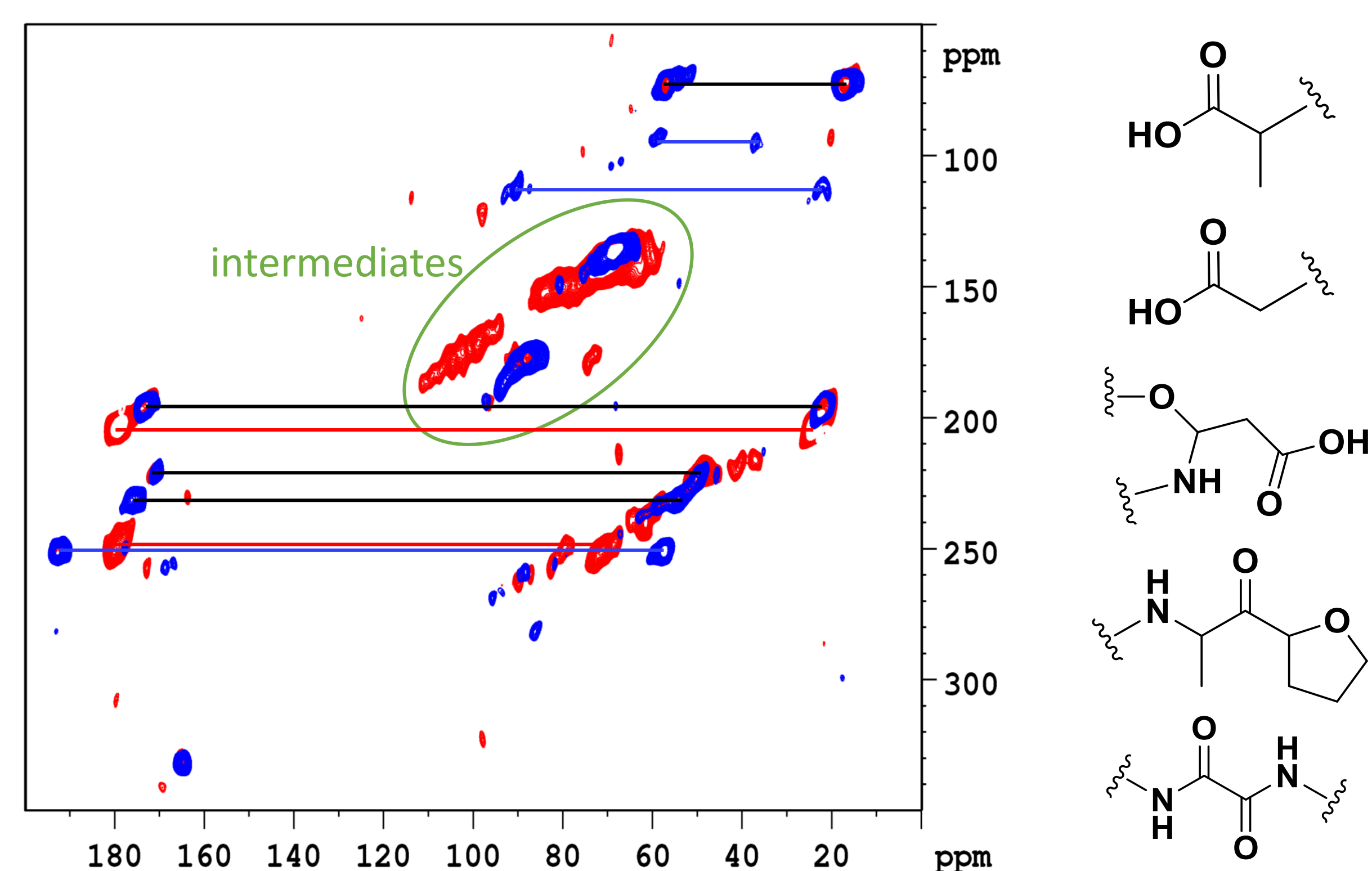


Figure 5. Comparison of 2D ¹³C-¹³C SQ-DQ POSTC7 spectra of ¹³C-R5P glycated ECM (red) and glycated collagen (blue) and structures of some possible fragments

CONCLUSIONS & FUTURE WORK

- Major glycation products are not cross links as widely believed but amino acid sidechain modifications and glycation intermediates.
- These are more likely to form on the collagen surface and in the gap zones.
- Formation and accumulation of those glycation products in ECM changes the mechanical properties, eg. flexibility and will certainly affect ECM biological functions, eg. ECM-cell interactions.
- Assign as many species as possible
- Glycation on different ECM proteins using different biogenic glycation agents
- Establish the relationship between glycation and other diseases, eg. cancer and Alzheimer's
- Eventually find inhibitors to reduce the effects of glycation and better ways to treat glycation diseases

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