

**P004** NMR studies of the inhibition of pepsin by peptide glyoxal inhibitors

**Cosgrove S., Rogers L. Hewage C. and Malthouse, J.P.G**

*Centre for Synthesis and Chemical Biology, School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Dublin 4, Ireland.*

Aspartyl proteases are required for the multiplication of the AIDS virus and for producing the amyloid protein which causes Alzheimer's disease. HIV protease inhibitors have been highly effective in treating Aids patients and it is hoped that potent inhibitors of the beta secretases will also prove effective in treating Alzheimer's disease. Therefore inhibitors of the aspartyl proteases have great therapeutic potential. We have shown that the peptide glyoxals are potent inhibitors of the thiol protease papain and of the serine proteases subtilisin and chymotrypsin. Using  $^{13}\text{C}$ -NMR we have been able to show that glyoxal inhibitors react reversibly with an active site nucleophile in these enzymes to form a tetrahedral adduct which is tightly bound by the enzyme. In the present work we synthesise  $^{13}\text{C}$ -enriched peptide glyoxals, we assess their inhibitor potency, and use  $^{13}\text{C}$ -NMR to examine how the inhibitors interact with the aspartyl protease pepsin. Z-Ala-Ala-[2- $^{13}\text{C}$ ]Phe-glyoxal was synthesised from [1- $^{13}\text{C}$ ]Phenylalanine which was converted to its methyl ester. This was then coupled with Z-Ala-Ala to give Z-Ala-Ala-[2- $^{13}\text{C}$ ]Phe-OMe which was hydrolysed to the free acid. This was converted to the diazoketone and transformed into Z-Ala-Ala-[2- $^{13}\text{C}$ ]Phe-glyoxal using dimethyldioxirane. We show that peptide glyoxal inhibitors can be potent inhibitors of pepsin and that pepsin only binds one of the four glyoxal forms (one non-hydrated, one fully hydrated and two partially hydrated forms).