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1. Synthesis of KDDE Tetrapeptide

1.1. Synthesis of Ac-Lys-Asp-Asp-Glu-NH_2 (KDDE)

The peptide was synthesized using standard Fmoc solid-phase peptide chemistry on a multiple peptide synthesizer (Tetras, Advanced ChemTech) on Sieber amide resin (2g, 1.2mmol). The protected amino acids, Fmoc-Lys(Boc)-OH, Fmoc-Asp(tBu)-OH, and Fmoc-Glu(tBu)-OH, as well as the solutions for coupling and deprotecting reactions were separately dissolved and arranged in different bottles of the instrument.

The peptide chain was assembled by sequential acylation (20 min. for coupling) with 'in situ' activated Fmoc-amino acids. The 'in situ' activations of Fmoc-amino acids (3 eq. compared to the resin amount) were carried out using uranium salts (HBTU, 2.7 eq., HOBT 3 eq.) and DIEA (6 eq.). Re-coupling and capping were automatically performed at every cycle. The Fmoc protecting groups were removed at every subsequent cycle by three treatments with 25% piperidine in DMF for 10 min.

The tetrapeptide was cleaved from the peptidyl-resin and deprotected in a single reaction with TFA:TA:phenol:water:TIS = 85:5:5:5 for 2 hours at room temperature (25°C). Precipitation and multiple washing with diethyl ether gave the final crude peptide (300 mg).

1.2. Analysis and Purification

HPLC analysis was performed on a HPLC equipped with a 168 diode array detector, a 507e auto injector and a 32 KARAT software package (Gold System from Beckmann Coulter, Fullerton, CA). This HPLC system was coupled with an Ion-Trap Mass Spectrometer (LCQ Fleet from Thermo Fisher, Waltham, MA). Column used for analytical HPLC: BetaBasic C18, 150 Å, 0.46 cm × 15 cm, 5 µm from Thermo Fisher. Column used for flash chromatography: C18 XBridge, BEH OBD, size 250 x 19 mm, 5 µm, 130 Å from Waters.

For purification, we used the same chromatography system configured in a preparative mode and still coupled to the Thermo Fisher LCQ Fleet Mass Spectrometer for assisted purification. We diverted a minimal part of the flow (0.5 ml/min) to the MS ion trap, to monitor in real time the m/z profile of the eluting fractions. The full amount of crude was purified with 4 subsequent injections and 165 mg of final purified peptide (purity> 95%) were obtained. The flow rate was maintained at 1 ml/min for analytical runs and at 10 ml/min for purifications. The wavelength of 214 nm was used to monitor this gradient. The gradient used for the crude analysis: from 2% to 35% B (0.1 % Trifluoroacetic acid, TFA in Acetonitrile and the remaining; A is 0.1 % TFA in water) in 44 min; for the preparative runs: from 2% to 22% B in 40 min.

2. NMR Spectra of KDDE

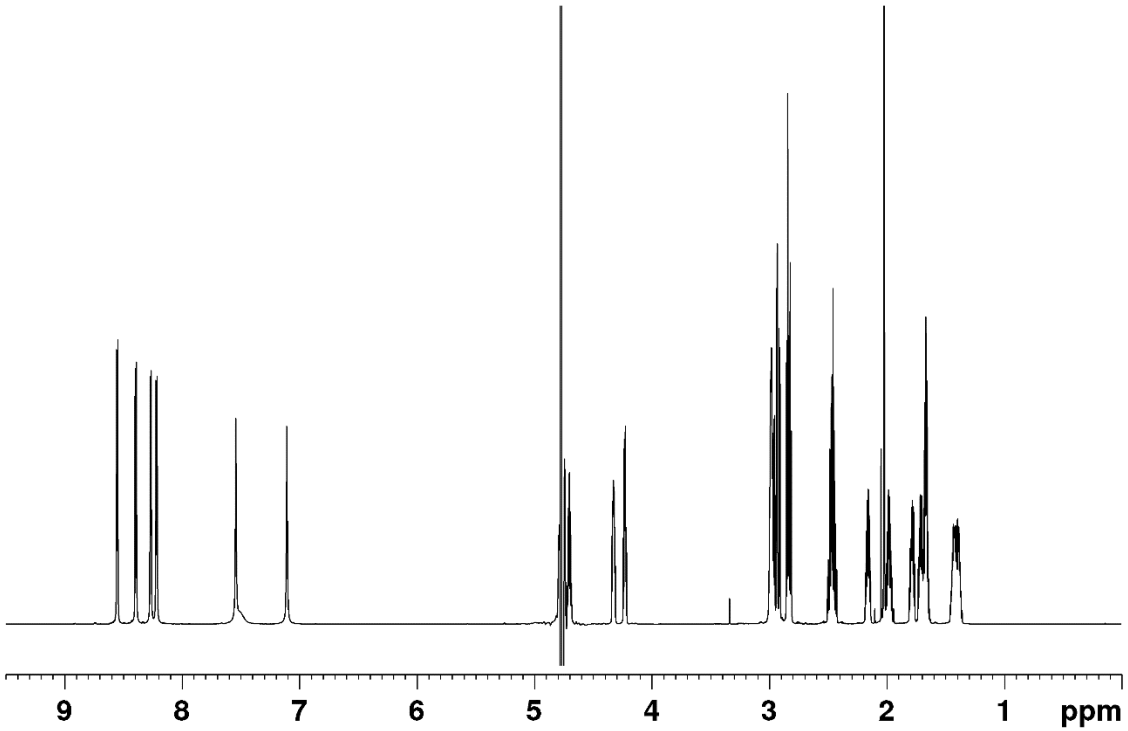

Figure S1. ^{1}H NMR of KDDE in 90% H_2O : 10% D_2O.

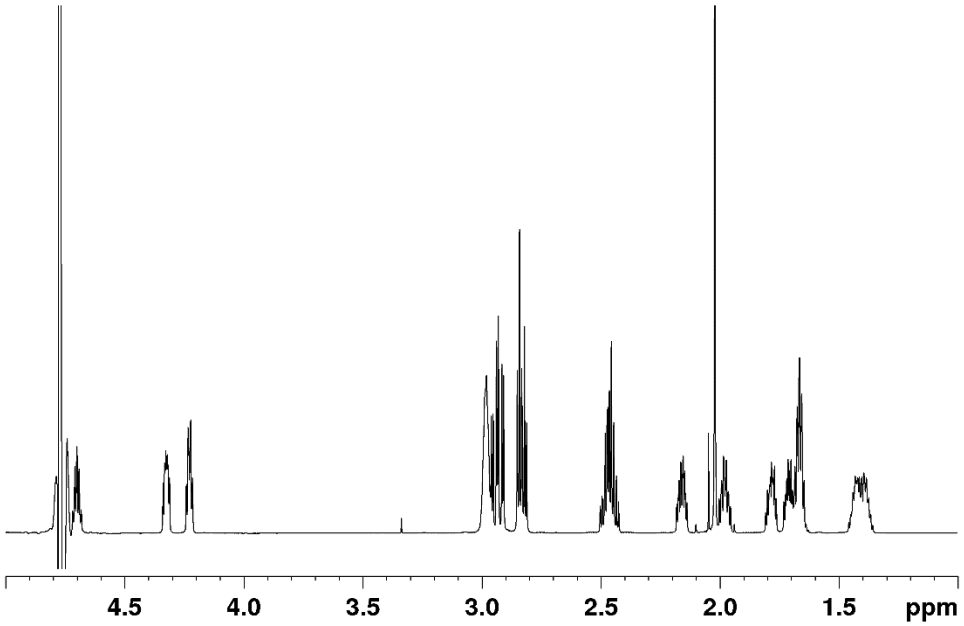

Figure S2. ^{1}H NMR of KDDE from 1 to 5 ppm in 90% H_2O : 10% D_2O.

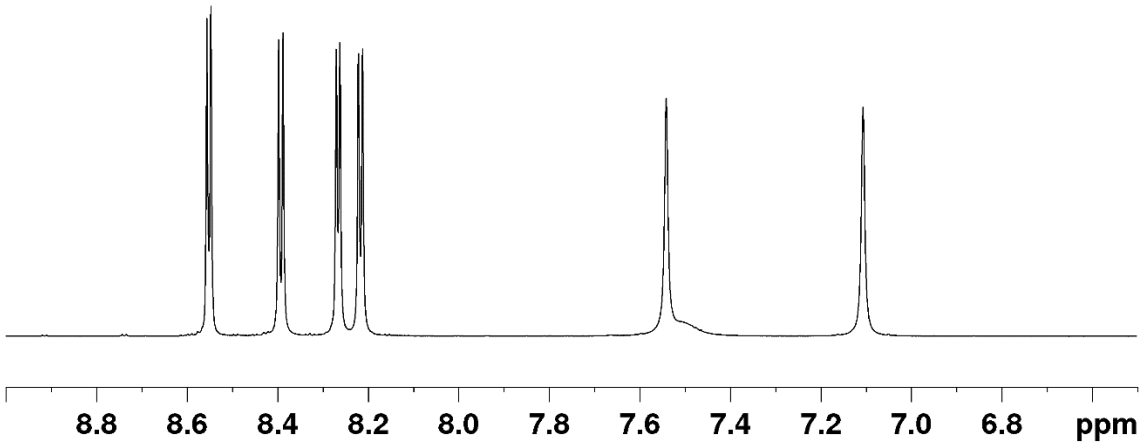

Figure S3. ^{1}H NMR of KDDE from 6.5 to 9 ppm in 90% H_2O : 10% D_2O.

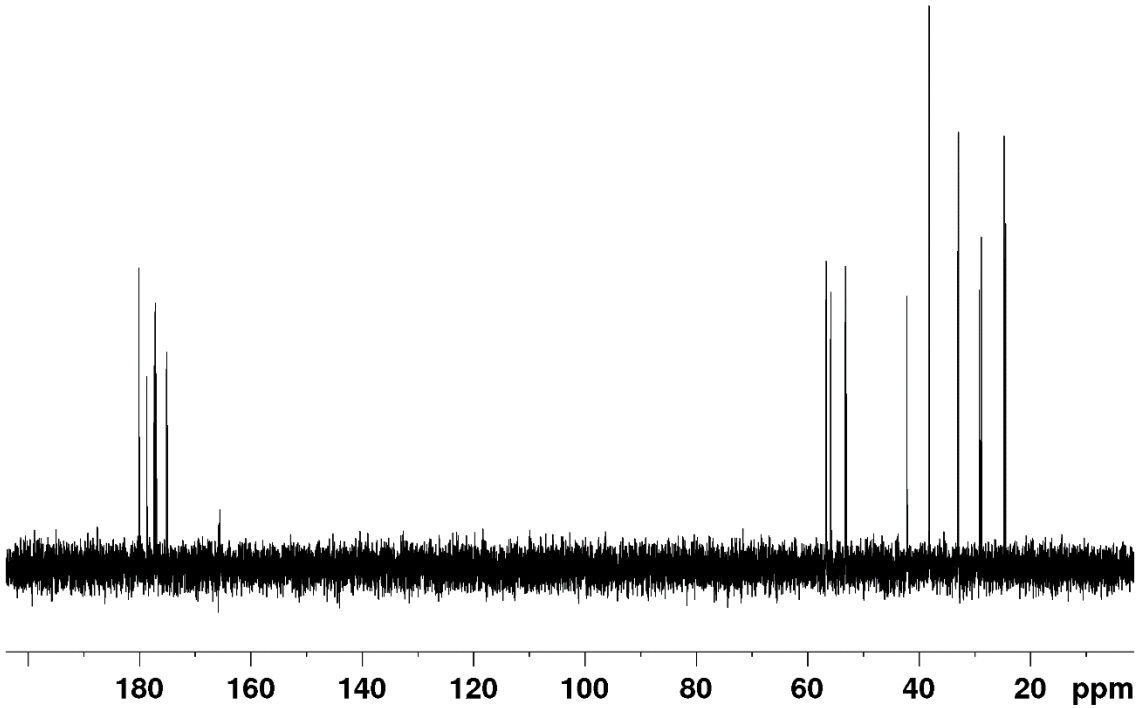

Figure S4. ^{13}C NMR of KDDE in 90% H_2O : 10% D_2O.

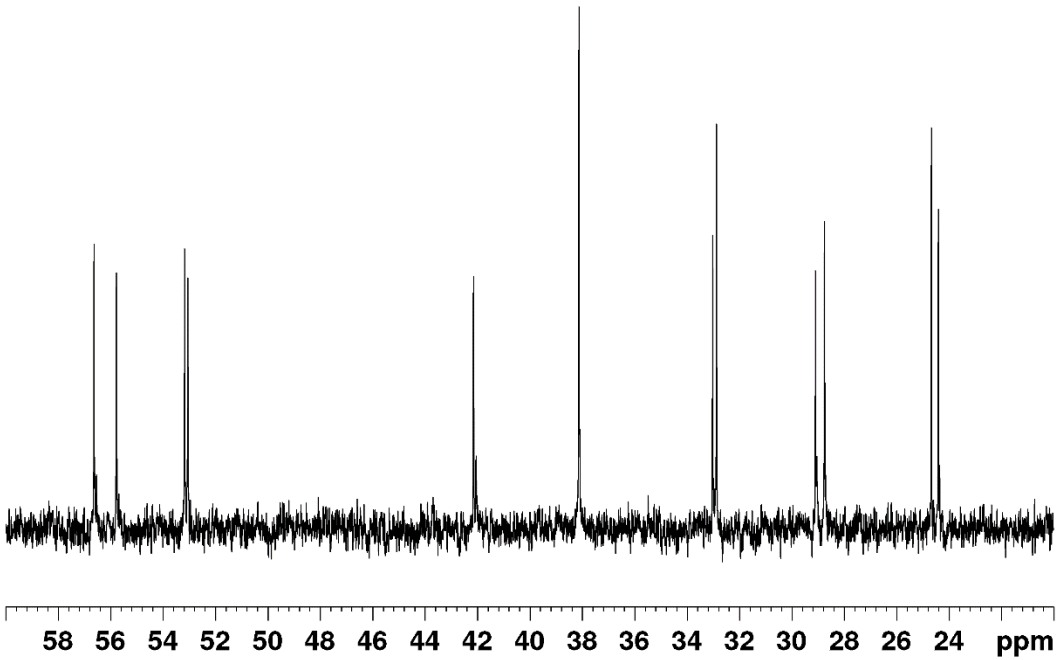

Figure S5. ^{13}C NMR of KDDE from 22 to 60 ppm in 90% H_2O : 10% D_2O.

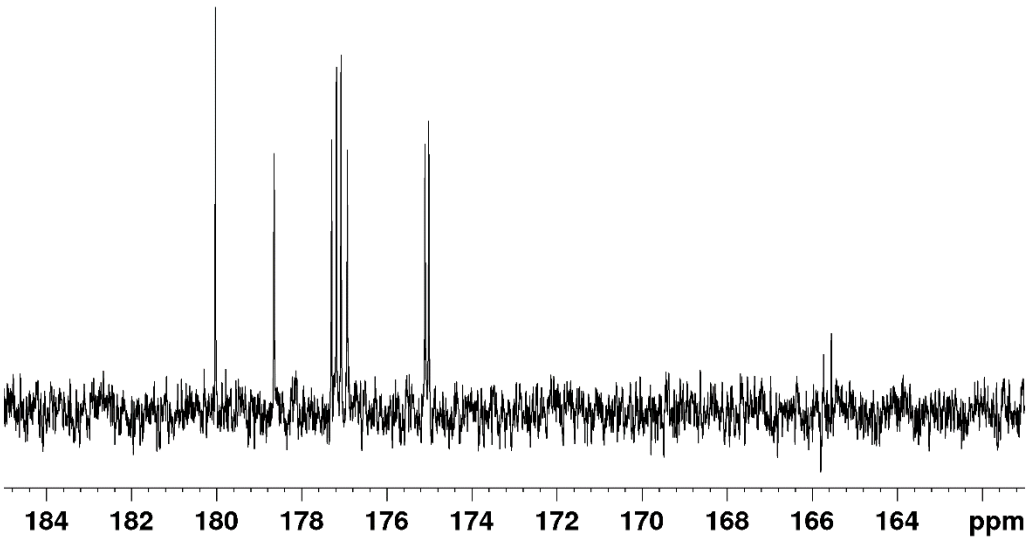

Figure S6. ^{13}C NMR of KDDE from 161 to 185 ppm in 90% H_2O : 10% D_2O.

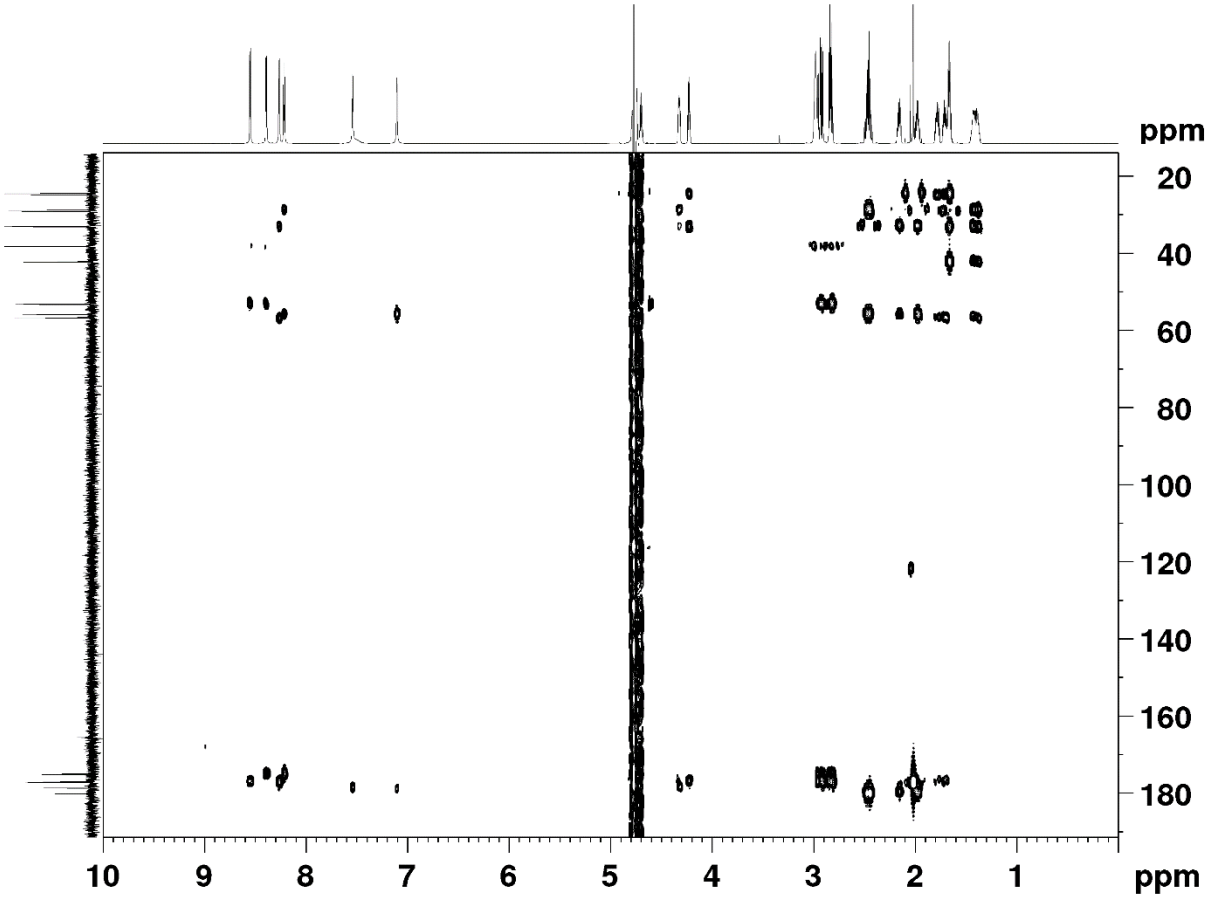

Figure S7. HMBC of KDDE in 90% H_2O : 10% D_2O.

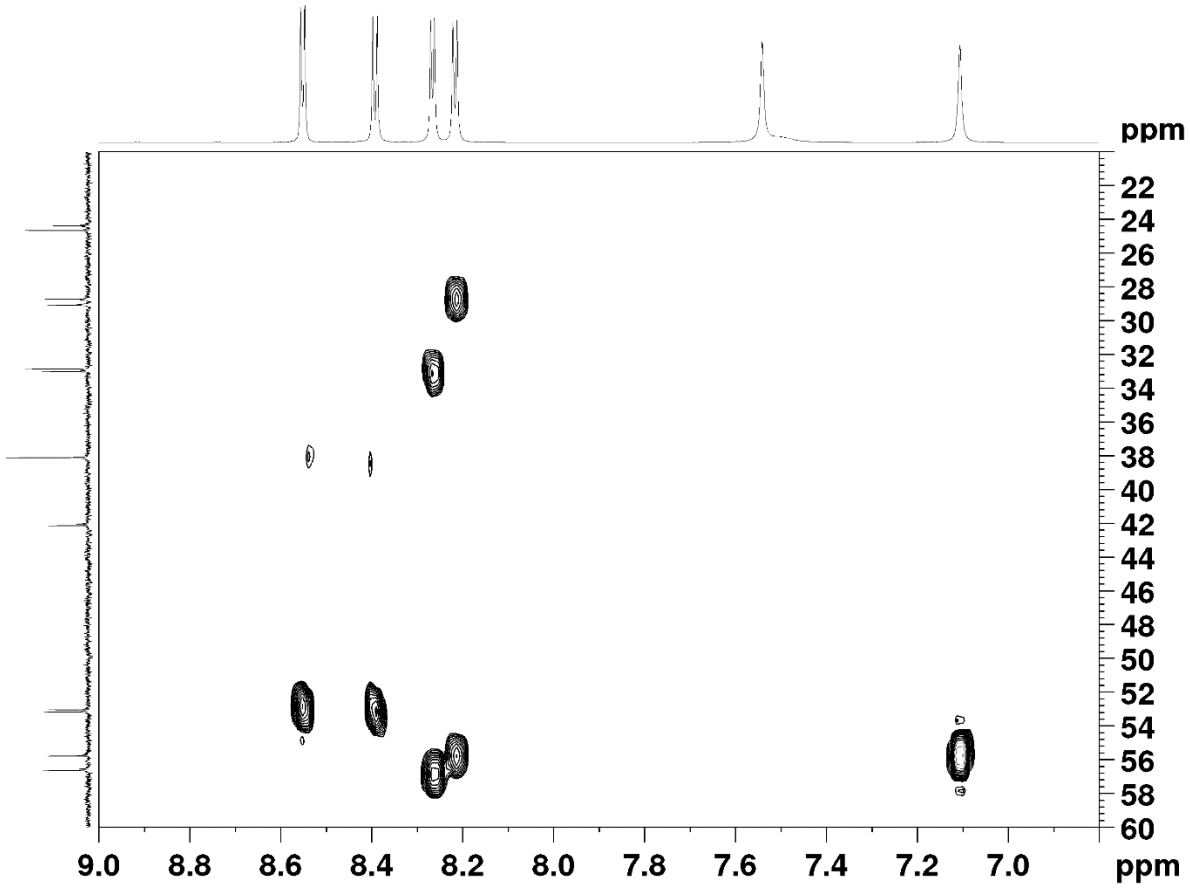

Figure S8. HMBC of KDDE in 90% H_2O : 10% D_2O from 6.8 to 9 ppm on x axes and from 20 to 60 on y axes.

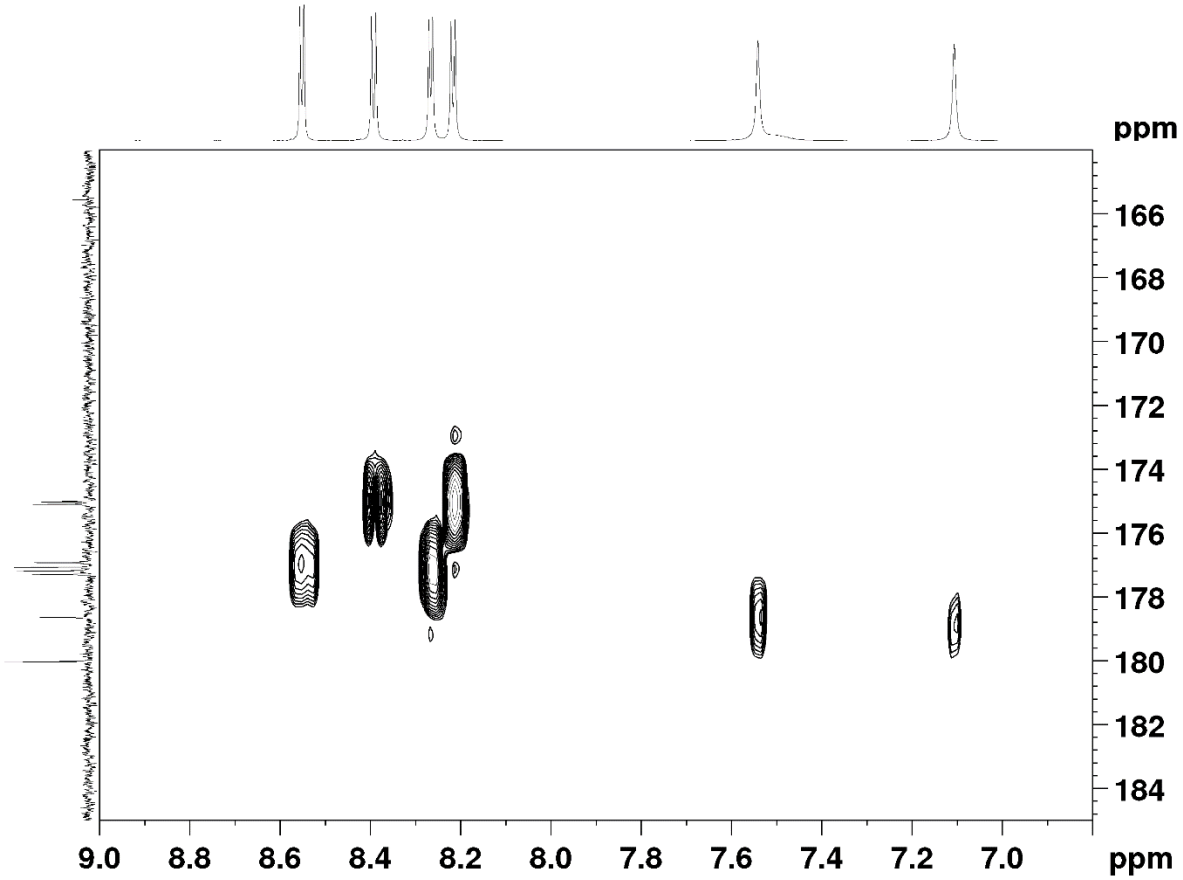

Figure S9. HMBC of KDDE in 90% H_2O : 10% D_2O from 6.8 to 9 ppm on x axes and from 164 to 185 on y axes.

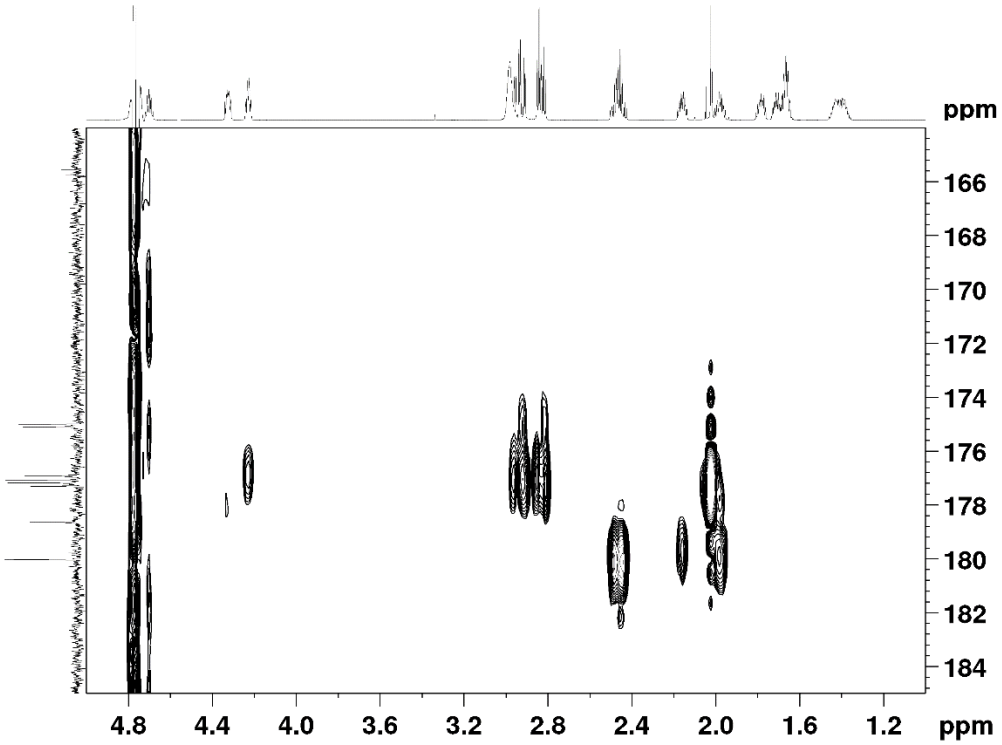

Figure S10. HMBC of KDDE in 90% H_2O : 10% D_2O from 1 to 5 ppm on x axes and from 164 to 185 on y axes.

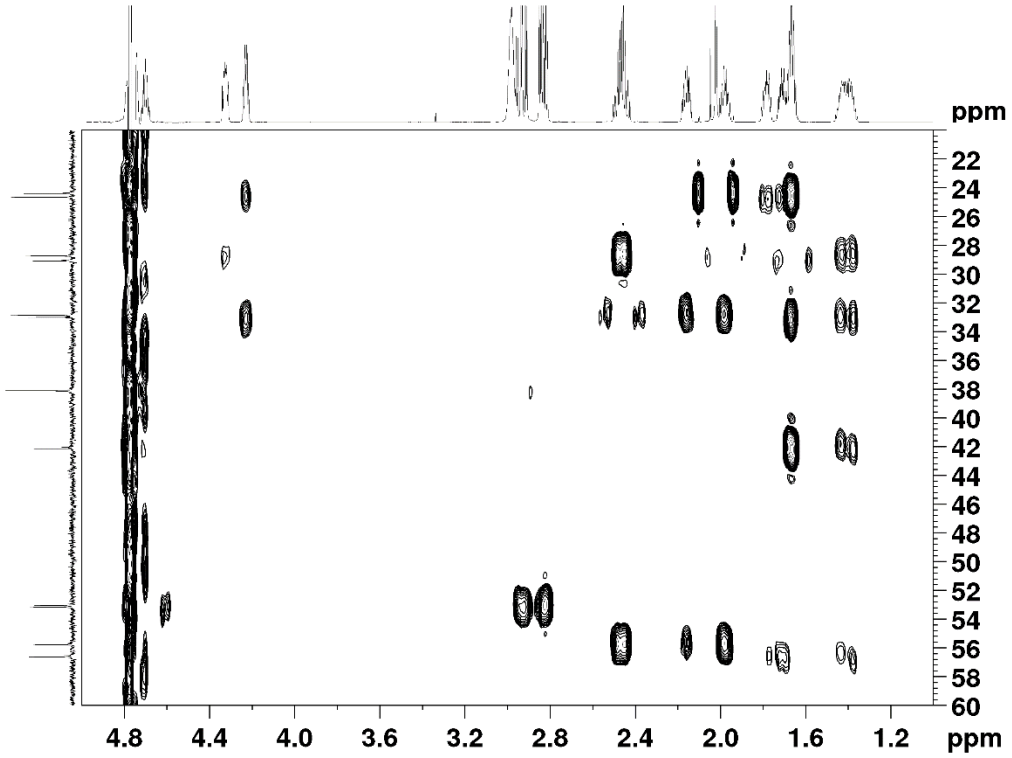

Figure S11. HMBC of KDDE in 90% H_2O : 10% D_2O from 1 to 5 ppm on x axes and from 20 to 60 on y axes.

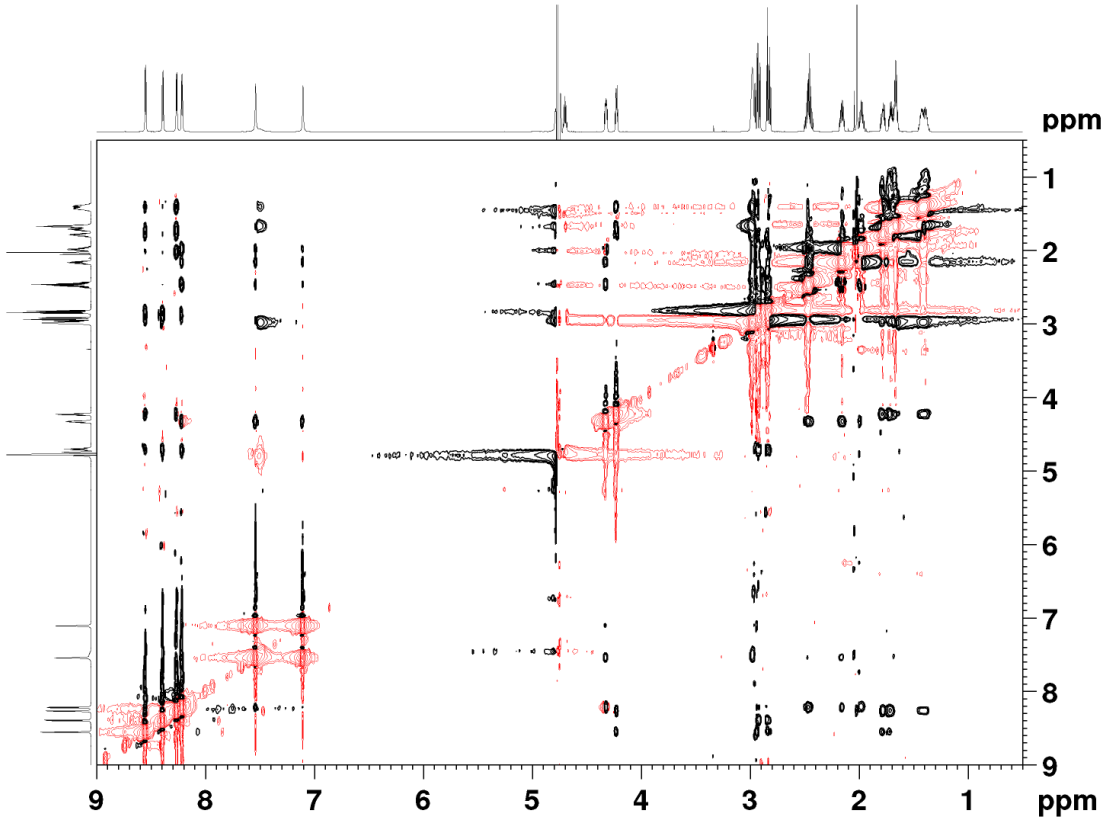

Figure S12. NOESY of KDDE in 90% H_2O : 10% D_2O.

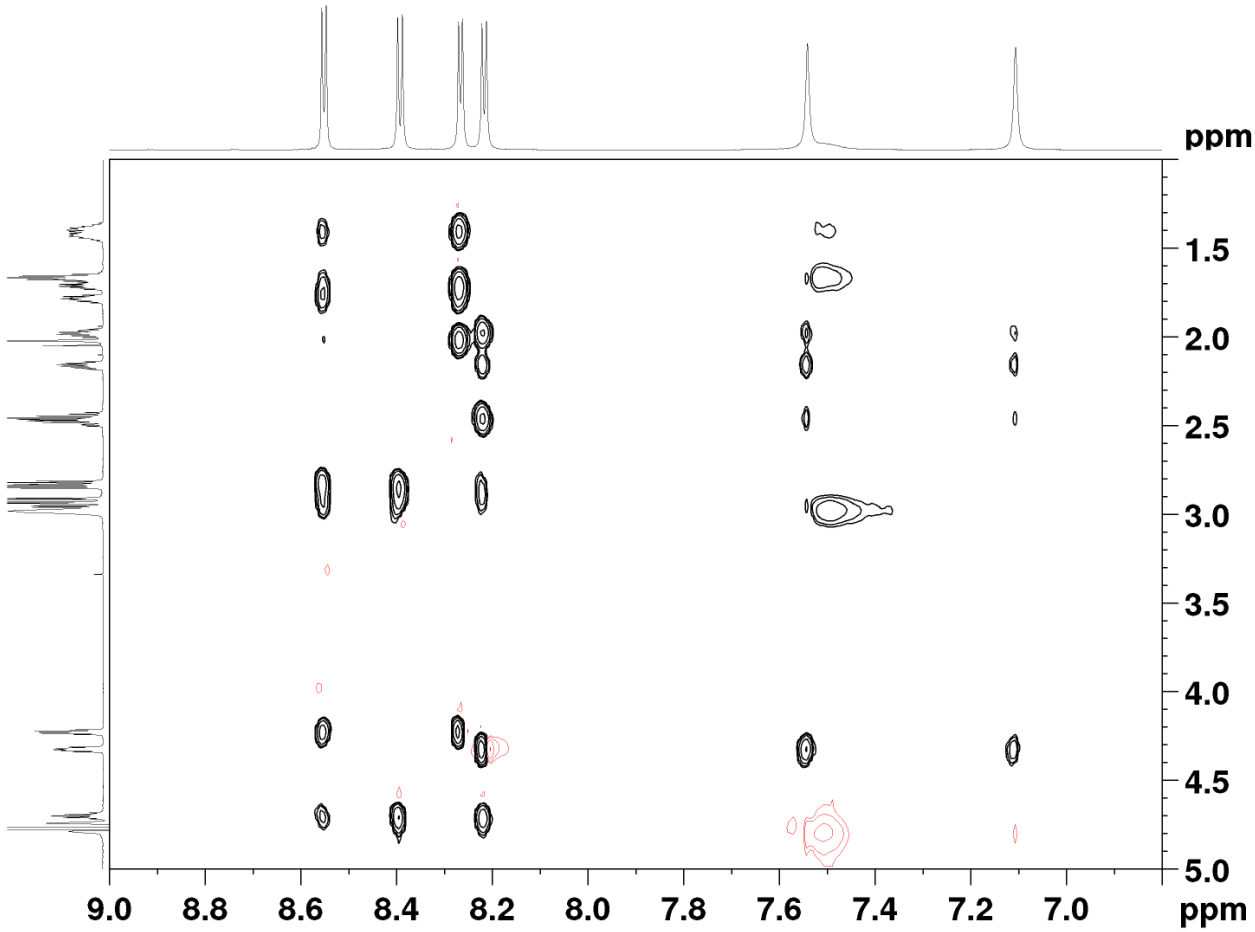

Figure S13. NOESY of KDDE in 90% H_2O : 10% D_2O from 6.8 to 9 ppm on x axes and from 1 to 5 on y axes.

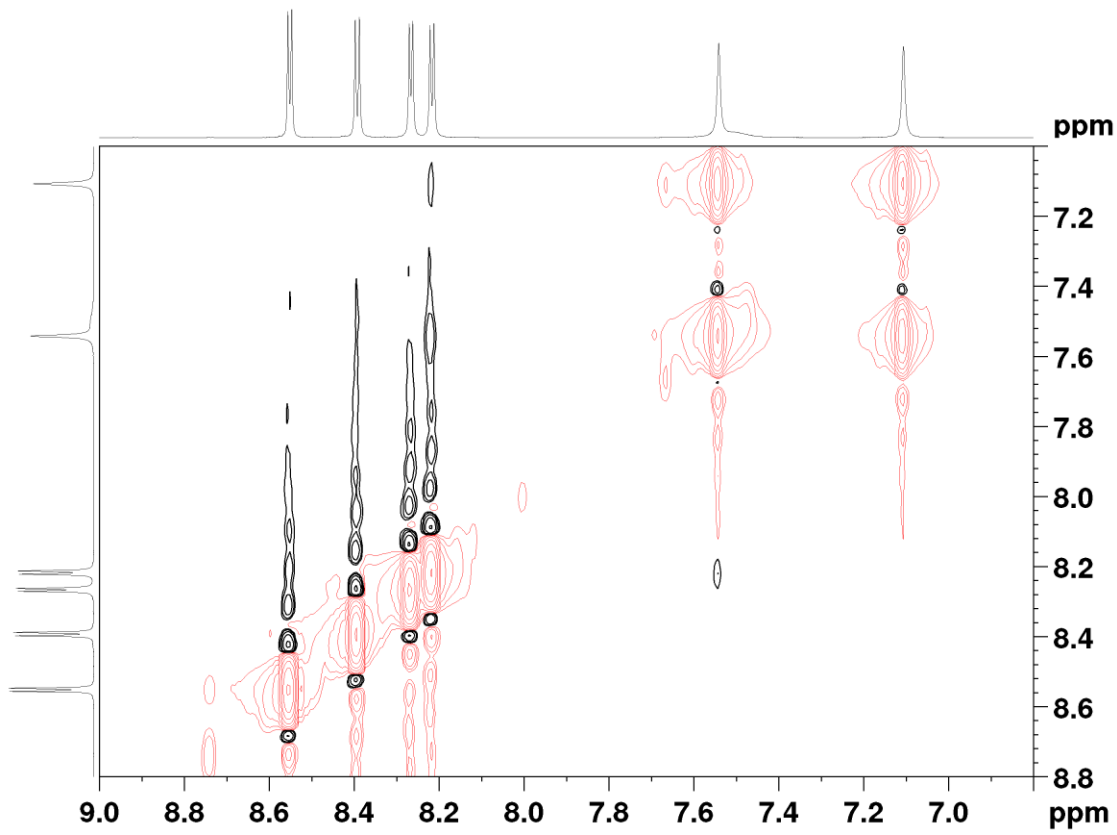

Figure S14. NOESY of KDDE in 90% H_2O : 10% D_2O from 6.8 to 9 ppm on x axes and from 7 to 8.8 on y axes.

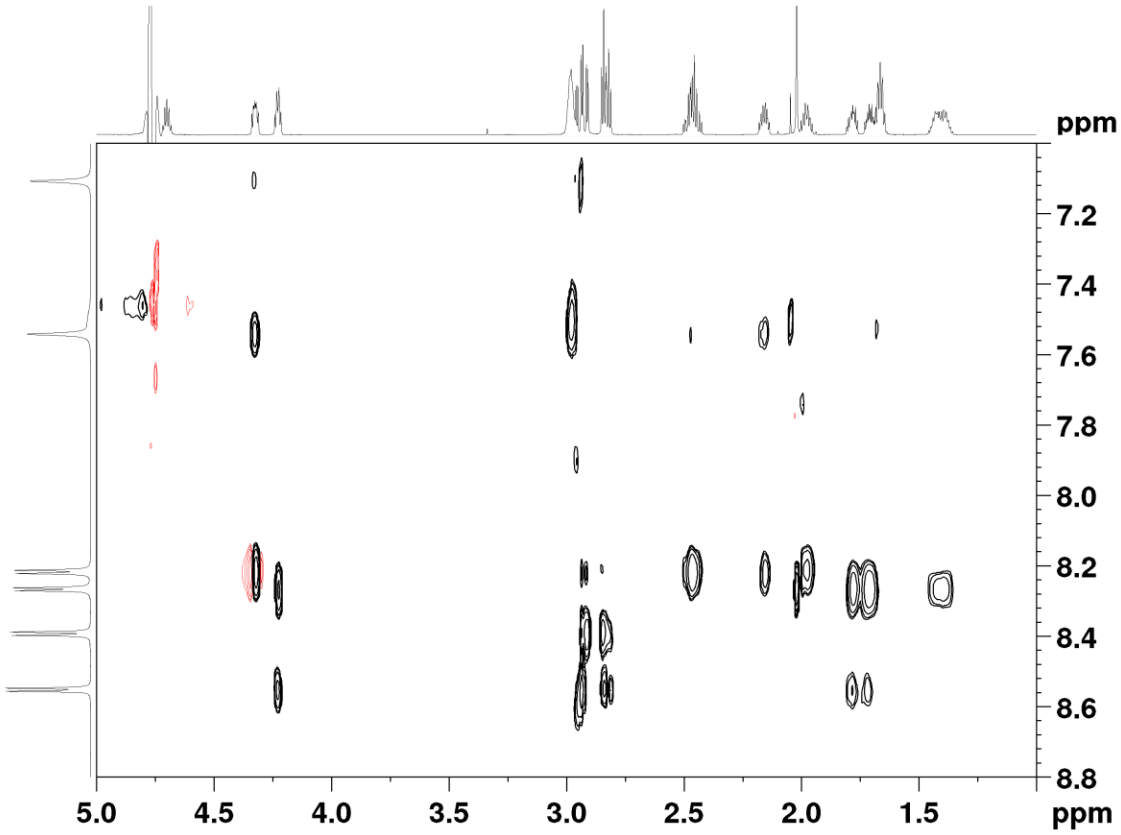

Figure S15. NOESY of KDDE in 90% H_2O : 10% D_2O from 1 to 5 ppm on x axes and from 7 to 8.8 on y axes.

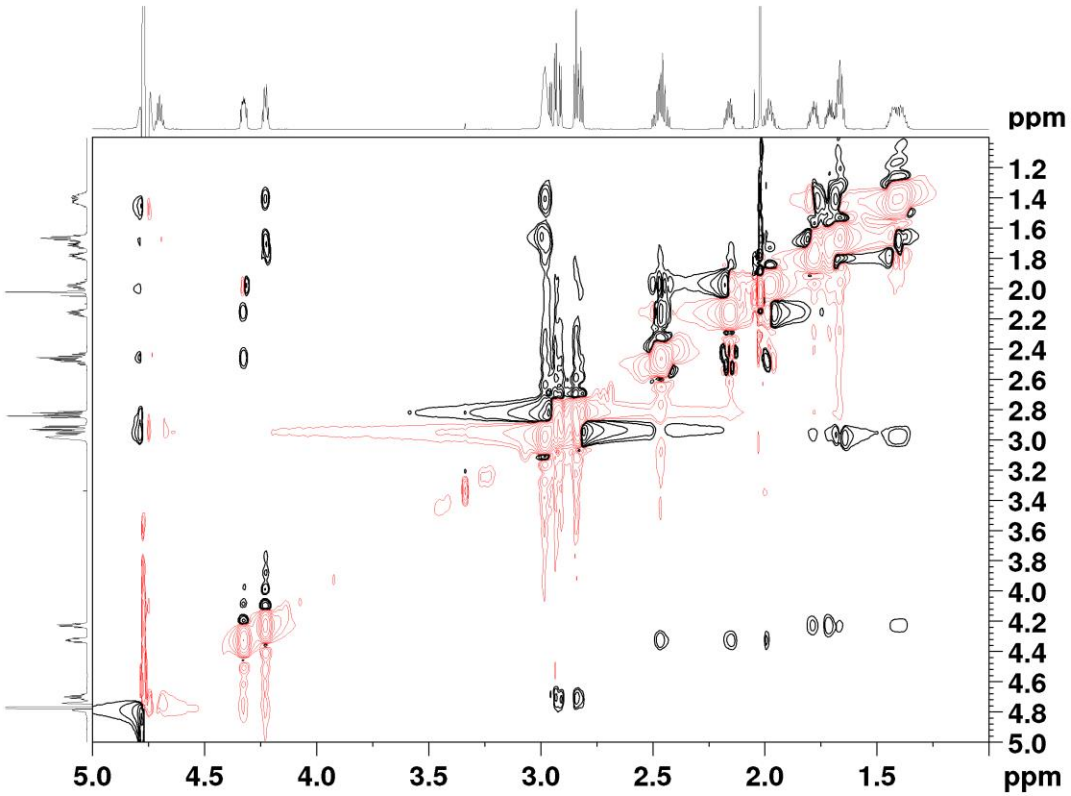

Figure S16. NOESY of KDDE in 90% H_2O : 10% D_2O from 1 to 5 ppm on x axes and from 1 to 5 on y axes.