

Supporting information

1. Detection limit probing of MAFC-proline

The detection limit of MAFC-proline was explored at pH 11 by mixing proline in H₂O with MAFC in ethanol in equimolar ratio at concentrations of 40 – 1000 μ M and measuring after 1 h. LOD/LOQ calculations revealed LOD at 11 μ M and LOQ at 38 μ M.

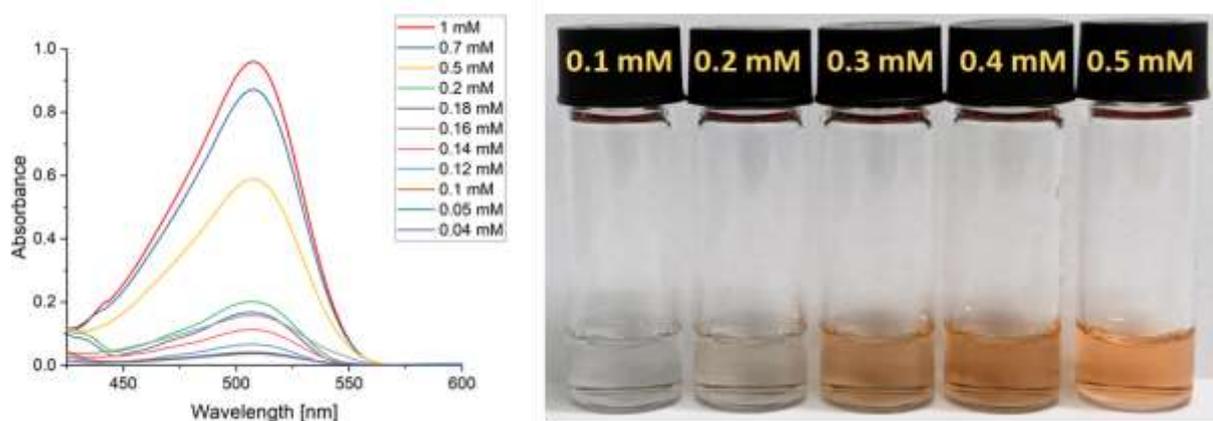


Figure S1: UV-Vis spectra of MAFC-proline at concentration of 40 – 1000 μ M and photo image of solutions showing increase of color intensity with increasing concentrations from 100 – 500 μ M

2. Time dependent UV-Vis analysis of MAFC-AA adducts at pH 11

UV-Vis measurements were performed by mixing amino acid (AA) and Meldrum's acid furfural conjugate (MAFC) at equimolar ratio in 1:1 water/ethanol solution to a final concentration of 0.3 mM. Time dependent studies of AA-MAFC adducts at pH 11 showed product stability and formation over 3 hours, after which the absorbance peaks at approximately 500 nm start decreasing and typical MAFC absorbance peak at 380 nm increases.

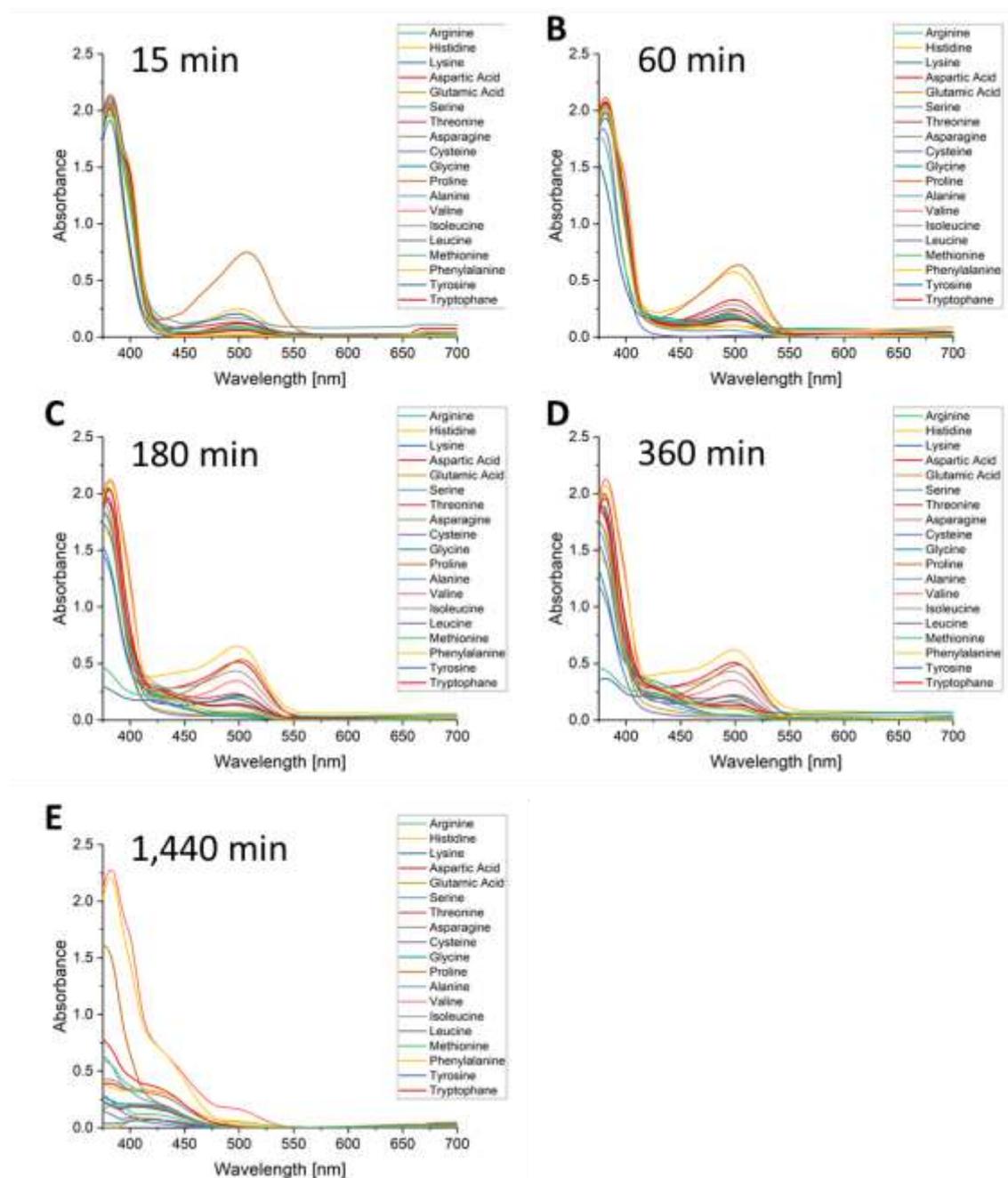


Figure S2: UV-Vis analysis of AA-MAFC adducts after A) 15 min, B) 1h, C) 3h, D) 6h, E) 24h reaction time, concentration of AA was 0.3 mM

3. $^1\text{H-NMR}$ of MAFC-proline

NMR measurements of MAFC-proline and α -hydroxyproline were done at equimolar ratio in 1:2 mixture of D_2O /Acetone- d_6 . To demonstrate the possibility of reaction between proline side chain functional group and MAFC pyrrolidine was chosen as chemical fragment of proline and reacted with MAFC following aforementioned protocol. Pure SH_2 in acetone- d_6 and proline in D_2O were measured as control. Peaks at 4.79 ppm, 6.03-6.44 ppm and 7.31-7.56 ppm clearly showed triene formation both in MAFC-proline and MAFC-pyrrolidine (see figure S3). Disappearing of MAFC typical peaks at 6.9 ppm, 8.2 ppm and 8.4 ppm indicate 1:1 reaction of the reactants. Specific proton assignments for the spectra can be found below (see figure S5-S7).

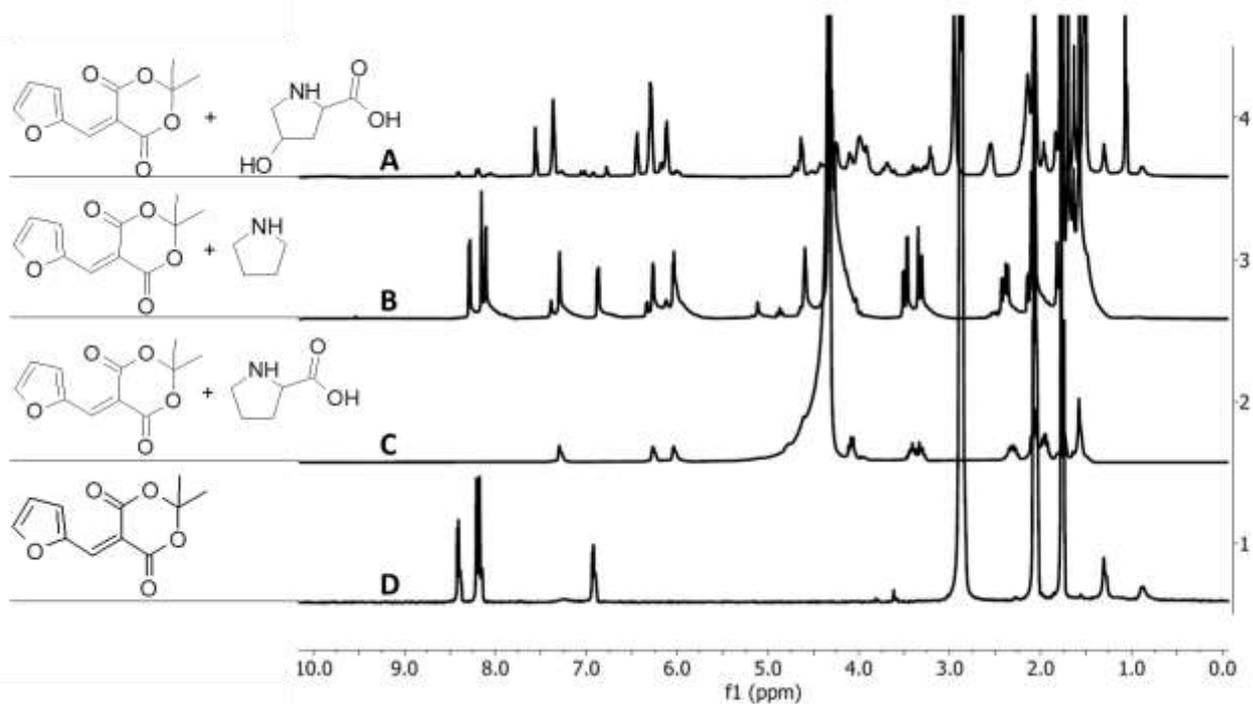


Figure S3: $^1\text{H-NMR}$ -spectrum of A) MAFC-hydroxyproline, B) MAFC-pyrrolidine, C) MAFC-proline and D) MAFC crude reaction mixture in acetone- d_6 / D_2O

To support our proposal of 1:1 addition reaction between MAFC and proline, different ratios of reactants were tested; namely 1:1, 2:1, 4:1 and 6:1. The NMR spectra clearly showed incomplete reaction through increasing signals of furan ring at 6.9 ppm, 8.2 ppm and 8.4 ppm of MAFC with increasing ratios (see figure S4).

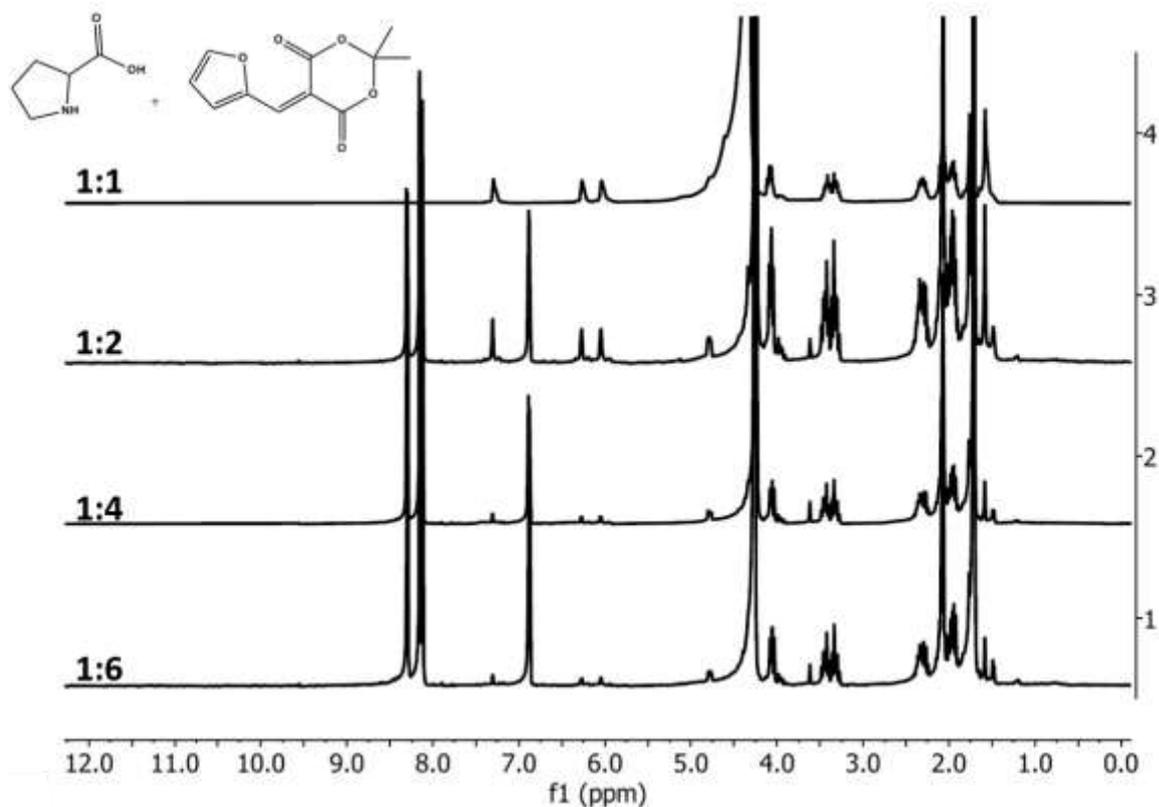


Figure S4: ^1H -NMR-spectra of MAFC-proline crude reaction mixture in different molar ratios of 1:1 - 6:1 in acetone- d_6 / D_2O

In the following single NMR spectra of MAFC-hydroxyproline, MAFC-proline, MAFC, proline and hydroxyproline are listed.

3.1 NMR of MAFC-hydroxyproline

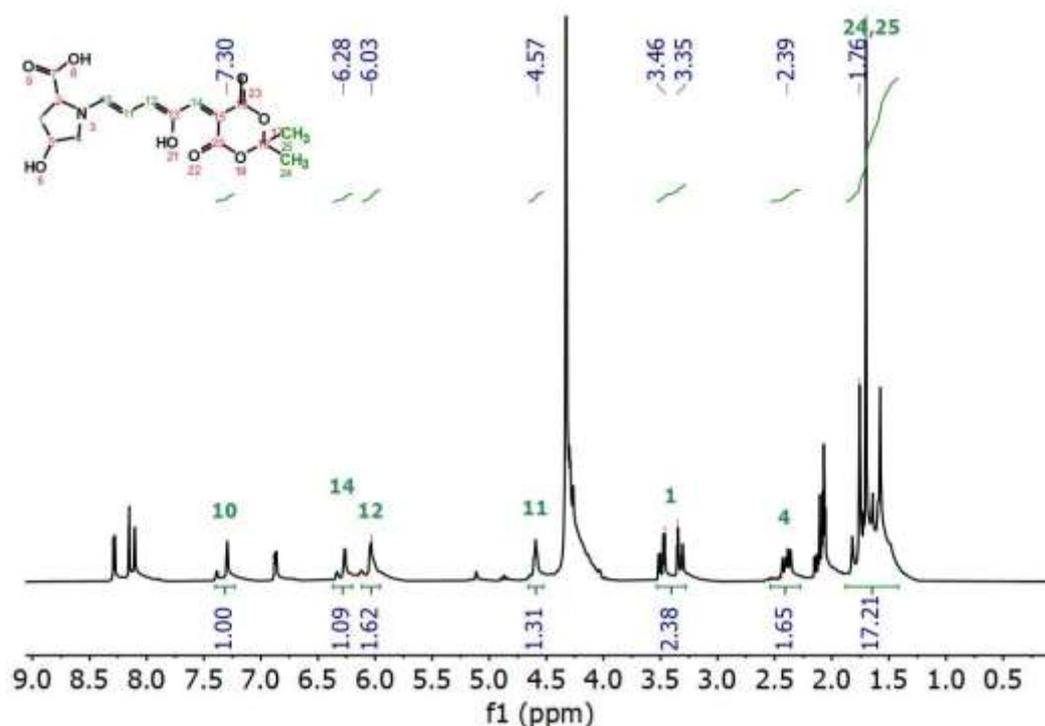


Figure S5: ¹H-NMR-spectrum of MAFC-hydroxyproline crude reaction mixture in acetone-d₆/D₂O

¹H-NMR (Acetone-d₆/D₂O): δ [ppm]: 7.3 (d, 1H, $J = 27$ Hz), 6.28 (s, 1H), 6.03 (d, 1H, $J = 3$ Hz), 4.57 (m, 1H), 3.46 – 3.35 (m, 2H), 2.39 (m, 2H, $J = 12$ Hz, $j = 9$ Hz), 1.76 (m)

The NMR spectrum of MAFC-hydroxyproline (see figure S5) showed an incomplete reaction after prolonged reaction time. This is assumed to be due to the decision of using acetone-d₆ as solvent instead of alkaline D₂O, which decreases reaction time and efficiency of the reaction tremendously but is more convenient regarding sample preparation. Peaks at 8.20 - 8.15 ppm depict an intact furan ring of MAFC while peaks at 7.30, 6.03 and 4.57 ppm indicate triene formation resulting from the ring opening reaction. Only peaks relevant to the final MAFC-hydroxyproline product are integrated and assigned.

3.2 NMR of MAFC-proline

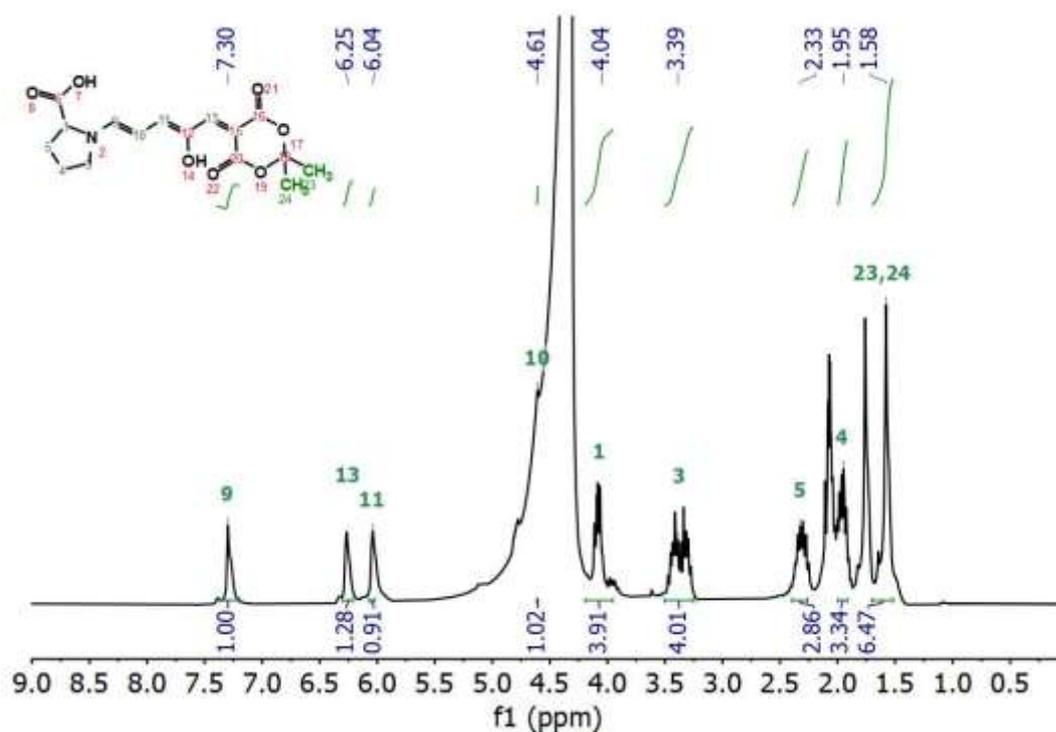


Figure S6: $^1\text{H-NMR}$ -spectrum of MAFC-proline crude reaction mixture in acetone- $d_6/\text{D}_2\text{O}$

$^1\text{H-NMR}$ (Aceton- $d_6/\text{D}_2\text{O}$): δ [ppm]: 7.3 (d, 1H, $J = 6$ Hz), 6.25 (s, 1H), 6.04 (d, 1H, $J = 3$ Hz), 4.61 (1H), 4.04 (dd, 4H, $J = 9$ Hz, $j = 6$ Hz), 3.39 (m, 4H), 2.33 (m, 2H), 1.95 (m, 3H, $J = 6$ Hz), 1.58 (s, 6H)

$^1\text{H-NMR}$ spectrum of MAFC-proline (see figure S6) clearly showed the triene formation of the DASA conjugate at 7.3, 6.04 and 4.61 ppm. No peaks indicating remaining furan species in the solution are visible. Non-assignable proton peaks are assumed to result from degradation products due to the necessary prolonged reaction time in acetone- $d_6/\text{D}_2\text{O}$ solvent. Only peaks relevant to the final MAFC-proline product are integrated and assigned.

3.3 NMR of MAFC

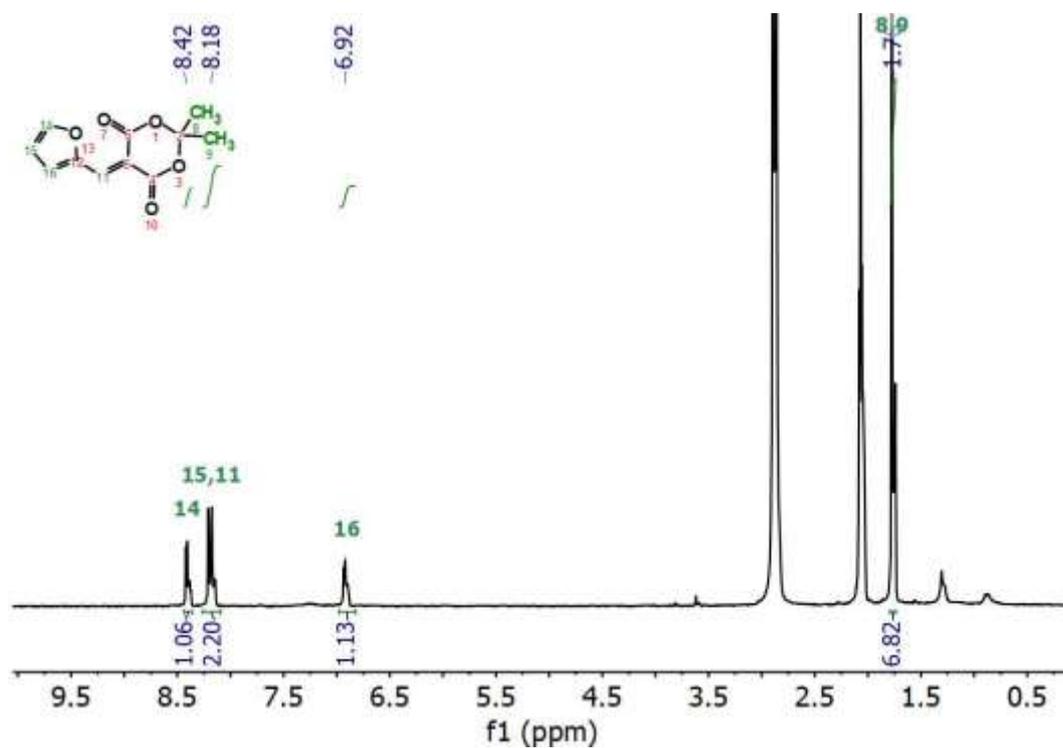


Figure S7: ¹H-NMR-spectrum of MAFC in acetone-d₆

¹H-NMR (Acetone-d₆): δ [ppm]: 8.42 (d, 1H, J = 3 Hz), 8.18 (dd, 2H, J = 9 Hz, j = 6 Hz), 6.92 (ddd, 1H, J = 3 Hz, j = 0.9 Hz), 1.76 (d, 6H, J = 9 Hz)

¹H-NMR spectrum showed purity of the synthesized MAFC product and typical Furan signals are seen at 8.42, 8.18 and 6.92 ppm (see figure S7).

3.4 NMR of proline

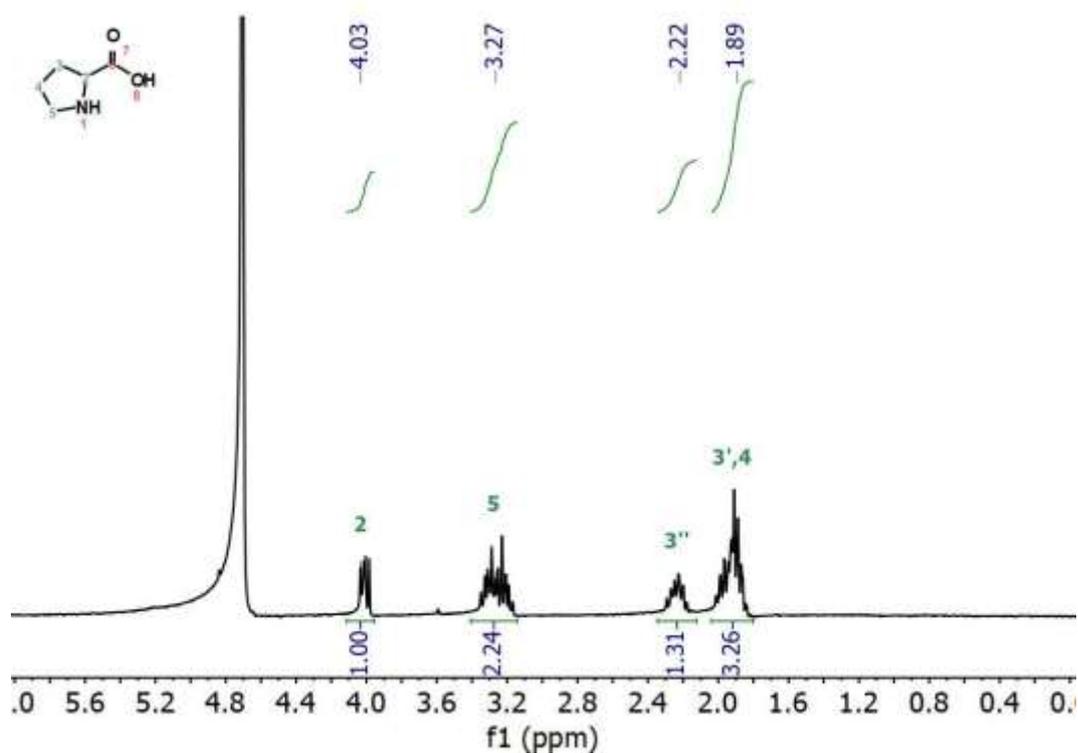


Figure S8: ^1H -NMR-spectrum of proline in D_2O

^1H -NMR (D_2O): δ [ppm]: 4.03 (dd, 1H, $J = 9$ Hz, $j = 6$ Hz), 3.27 (m, 2H), 2.22 (m, 1H), 1.89 (m, 3H)

Proline could be identified clearly without any impurities through ^1H -NMR (see figure S8).

3.5 NMR of hydroxyproline

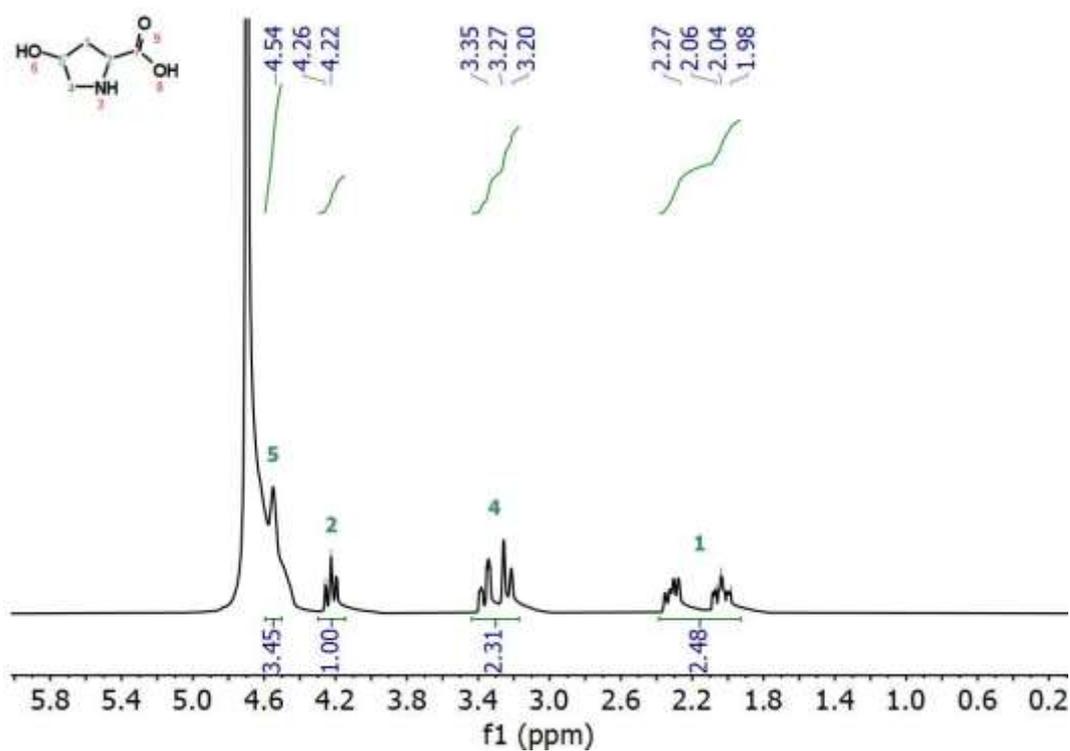


Figure S9: ¹H-NMR-spectrum of hydroxyproline in D₂O

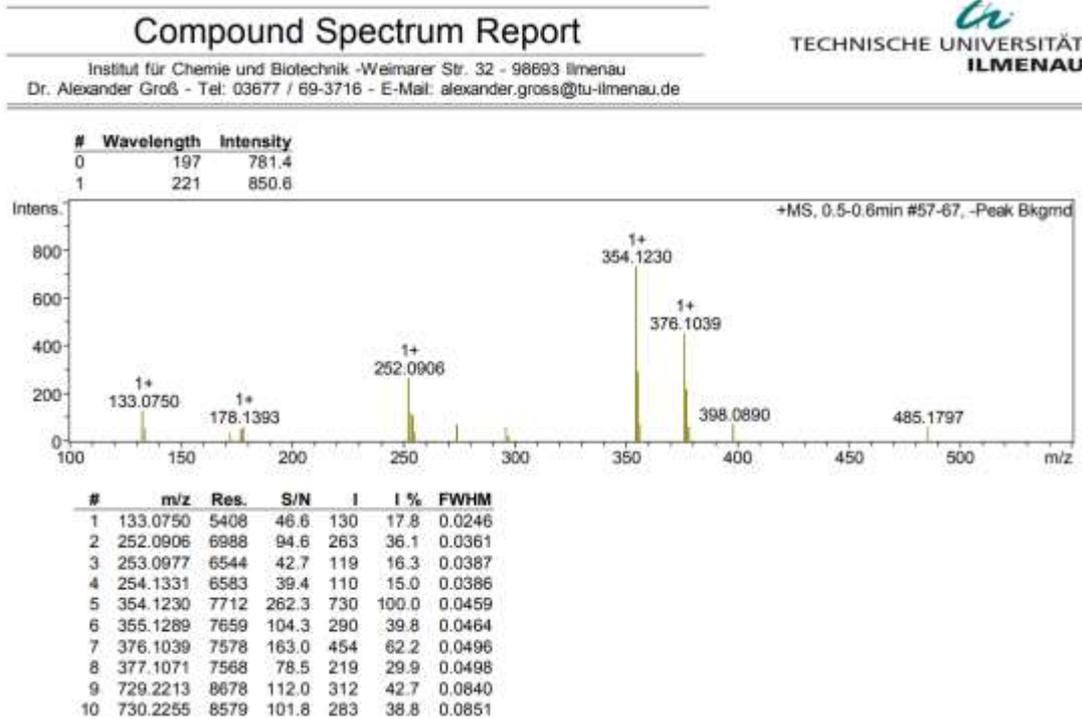
¹H-NMR (D₂O): δ [ppm]: 4.56 (s but superimposed due to solvent peak), 4.26 - 4.22 (m, 1H, J = 9 Hz), 3.35 - 3.2 (dd, 2H, J = 39 Hz, j = 12 Hz), 2.27 - 1.98 (dm, 2H)

As for proline, hydroxyproline that was used for experiments could be identified clearly without impurities that could impact the reaction with MAFC (See figure S9).

4. LCMS

LCMS analysis were done to prove our hypothesis for 1:1 adduct formation in MAFC and AA reaction. Product peaks of both MAFC-hydroxyproline and MAFC-proline at 354⁺ (MH⁺) and 338⁺ (MH⁺) respectively clearly establish evidence for 1:1 addition reaction products.

MAFC-hydroxyproline



MAFC-proline

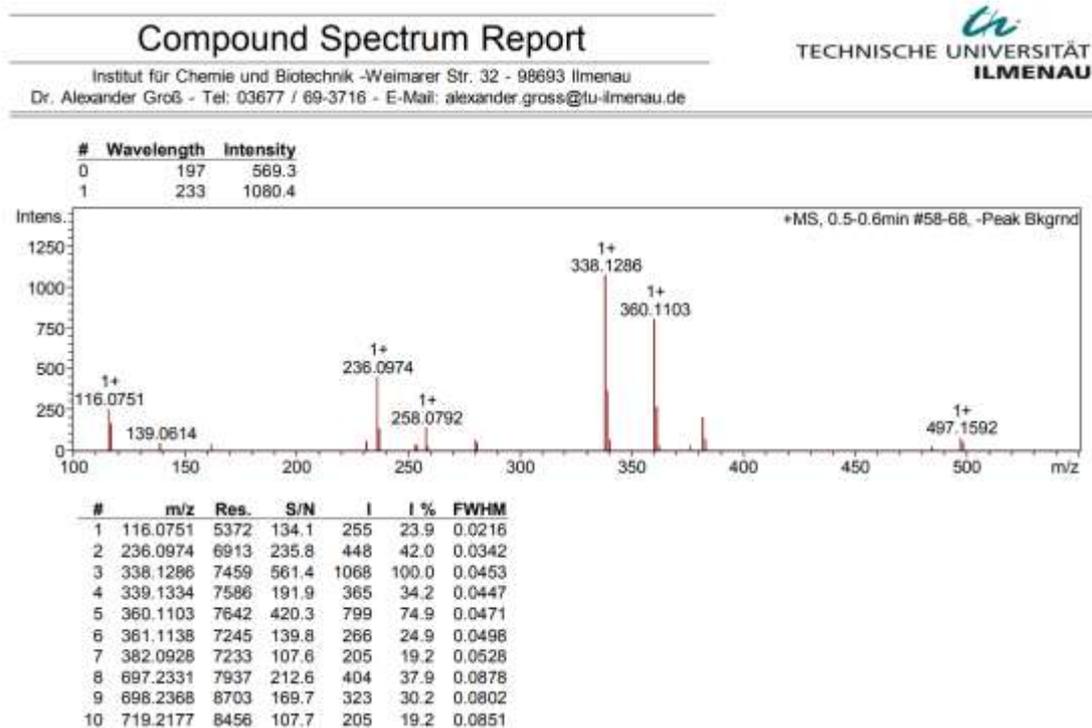


Figure S10: LCMS of MAFC-proline (top) and MAFC-hydroxyproline reaction (bottom)