

Furfurylamines from Biomass: Transaminase catalysed upgrading of furfurals

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Analytical methods

Achiral methods:

Analytical reverse phase analysis was used for quantitative analysis of enzymatic reactions with respect to chemical standards, using an Agilent 1260 Infinity HPLC with an Ace 5 C18 column 150 x 4.6 mm. Elution was carried out at 1 mL/min with a linear gradient of acetonitrile/H₂O containing 0.1% TFA, with detection at 250 or 210 nm, injection volume of 10 µL and column temperature of 30 °C. Chromatograms are included below.

Method A:

Products were detected with a linear gradient 15 – 72% acetonitrile over 15 min with detection at 250 nm.

This method was used to detect acetophenone (RT 11.5 min) and 5-aminomethyl-2-furancarboxylic acid **8b** (RT 1.9 min).

Method B:

Products were detected using a linear gradient 15 – 72% acetonitrile over 15 min with detection at 210 nm.

This method was used to detect benzylamine (RT 3.8 min) and 5-hydroxymethylfurfurylamine **2b** (2.6 min).

Method C:

Products were detected using a linear gradient 5 – 95% acetonitrile over 14 min with detection at 210 nm.

This method was used to detect furfurylamine **1b** (RT 2.9 min) and 2,5-bis(aminomethyl)furan **9b** (RT 2.2 min).

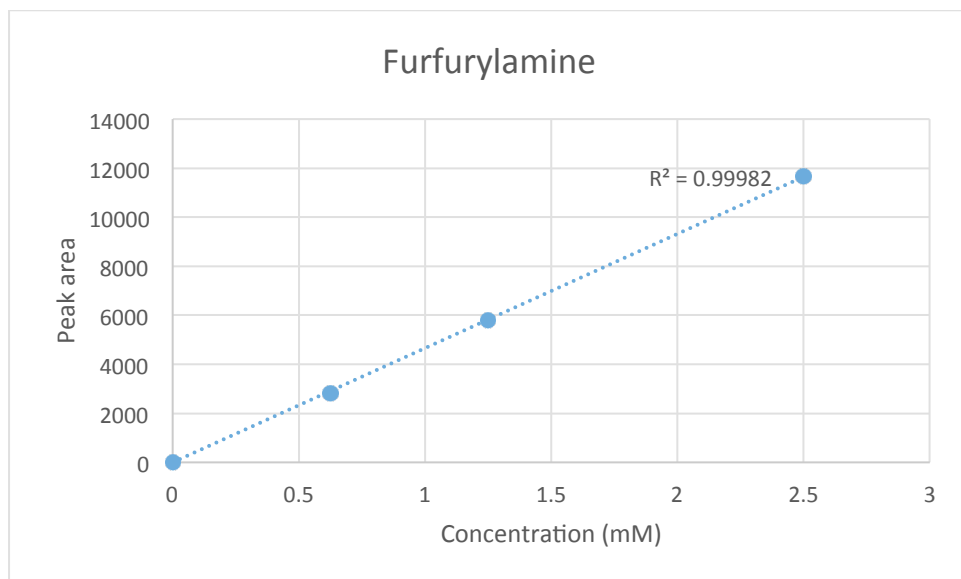
Chiral method:

Chiral analytical reverse phase analysis was used for determination of enantiomeric excess of CBz protected **10b** with respect to a Cbz protected commercial racemic sample, using a Hewlett Packard Series 1100 HPLC with a Chiralcel OD Column 250 x 4.6 mm. Elution was carried out at 0.5 mL/min isocratic flow of 4% 2-propanol/hexane for 50 min, injection volume of 10 µL and UV detection at 250 nm.

Calibration curves

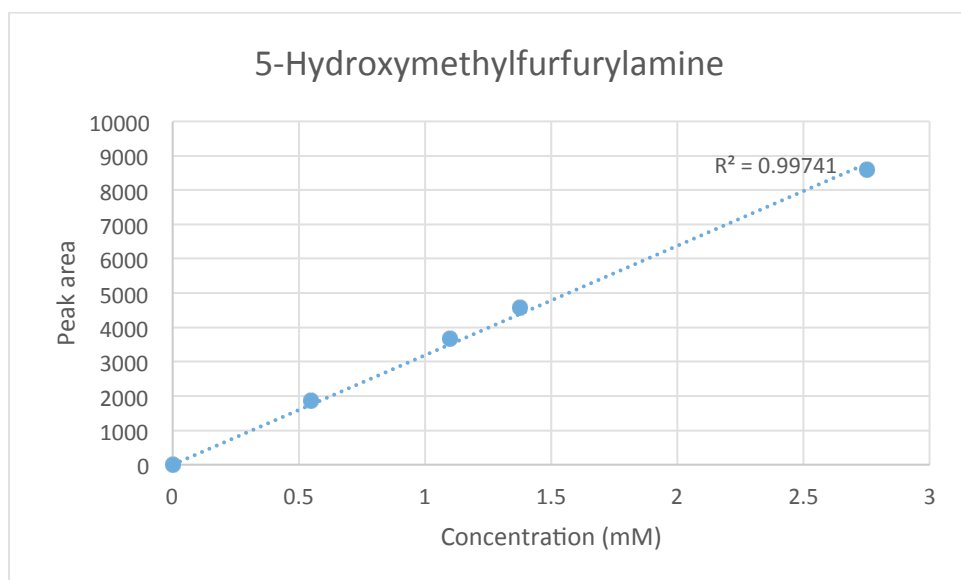
Furfurylamine **1b**

HPLC Method C, detection at 210 nm



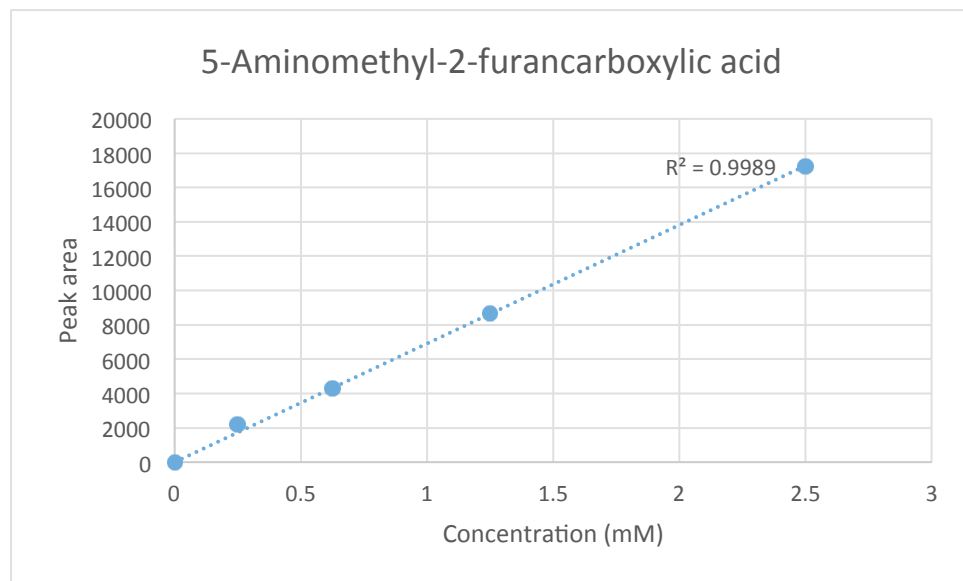
5-Hydroxymethylfurfurylamine **2b**

HPLC Method B, detection at 210 nm



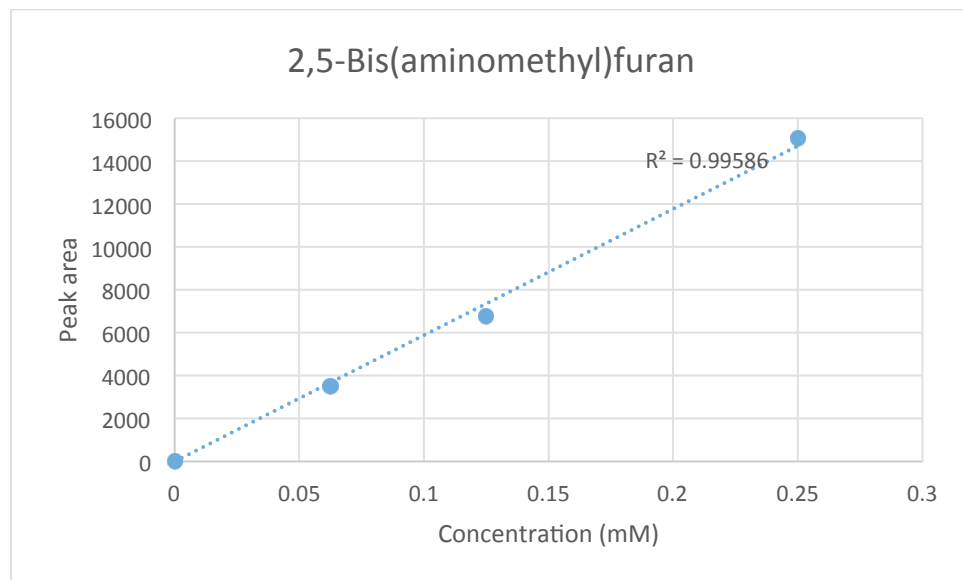
5-Aminomethyl-2-furancarboxylic acid **8b**

HPLC Method A, detection at 250 nm



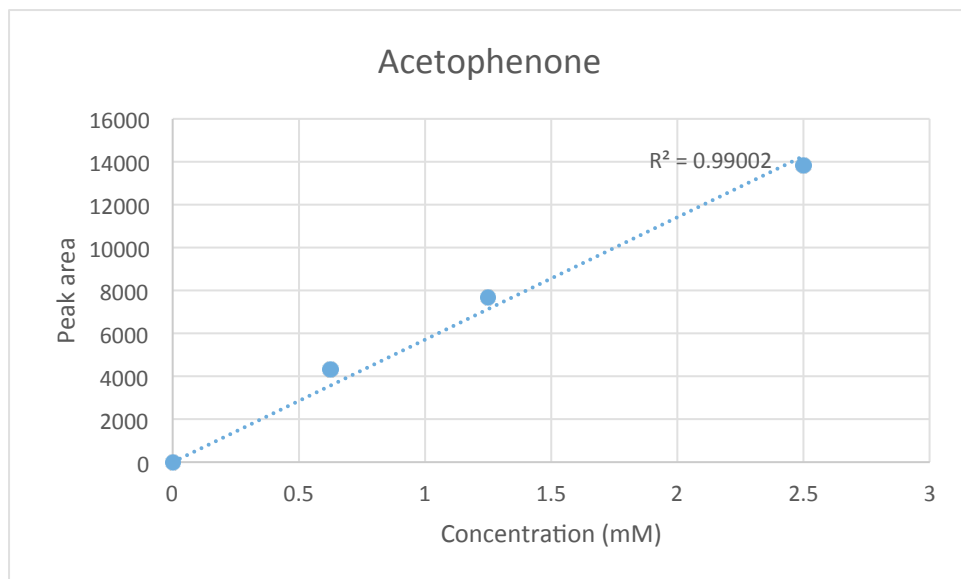
2,5-Bis(aminomethyl)furan **9b**

HPLC Method C, detection at 210 nm



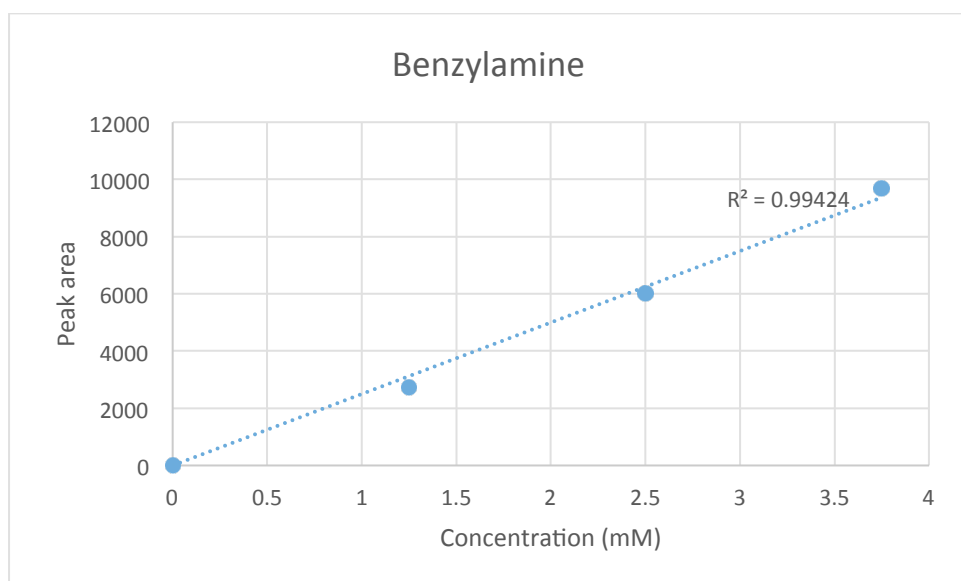
Acetophenone

HPLC method A, detection at 250 nm



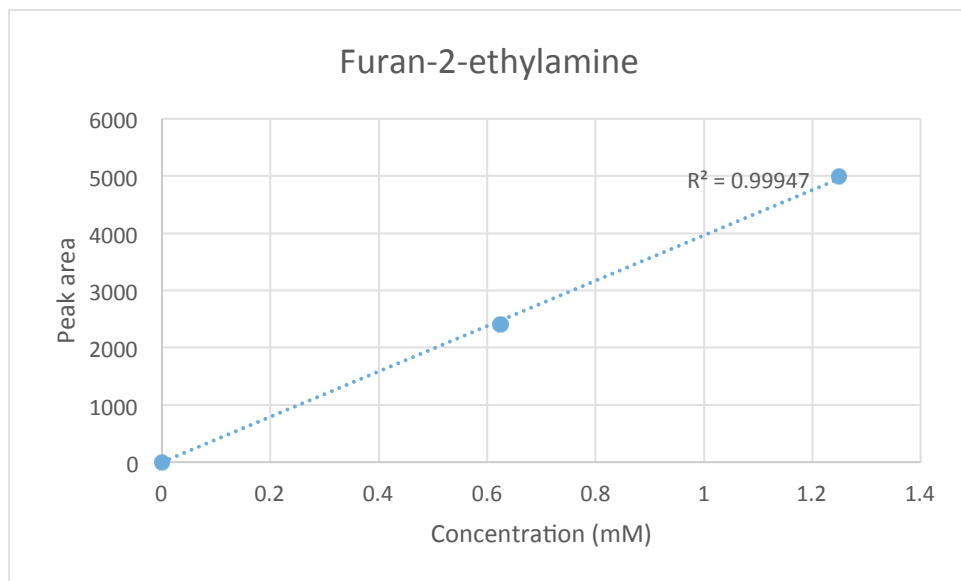
Benzylamine

HPLC Method B, detection at 210 nm



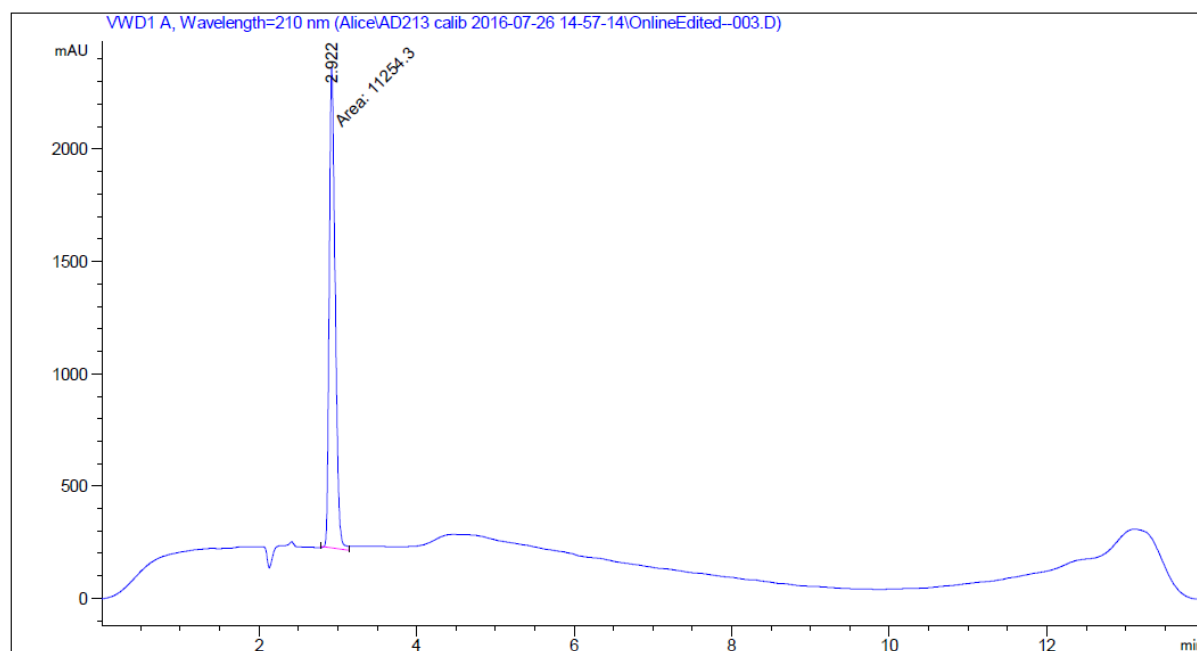
Furan-2-ethylamine **10b**

HPLC Method B, detection at 210 nm

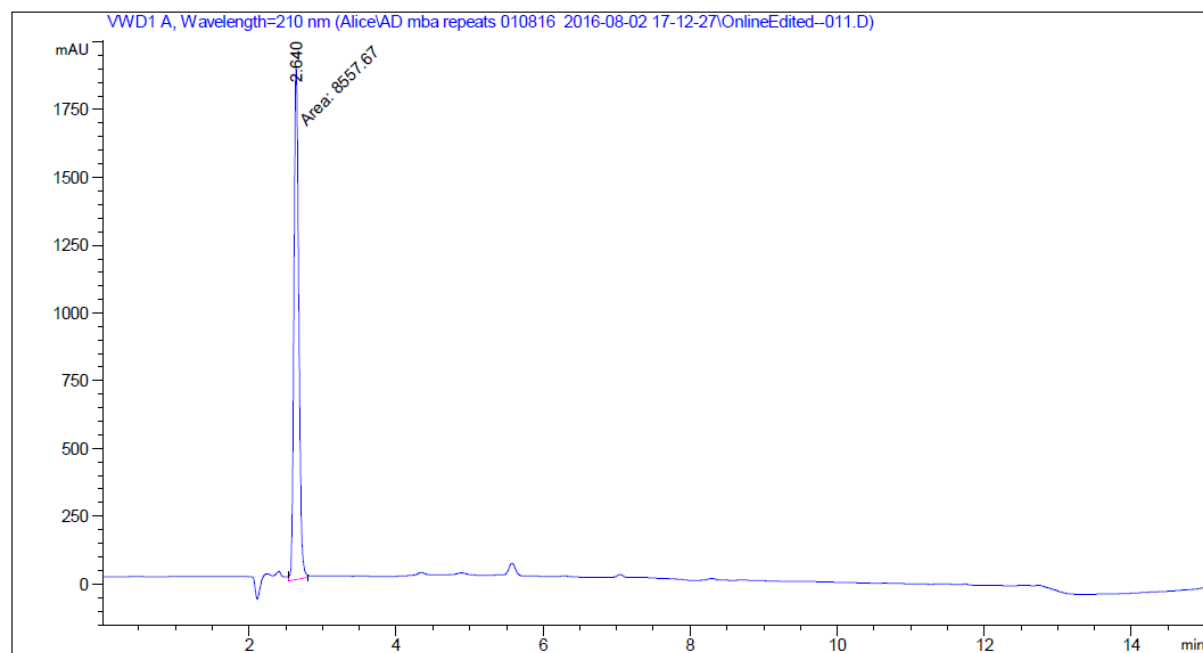


HPLC Chromatograms

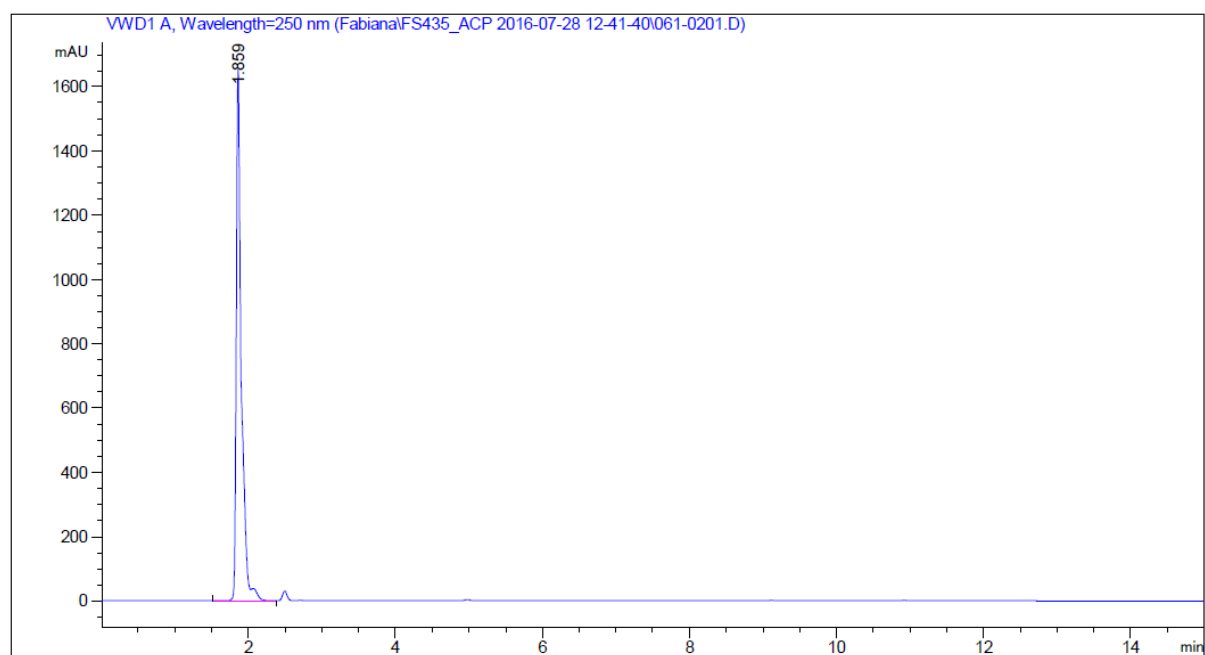
Furfurylamine **1b** (HPLC Method C)



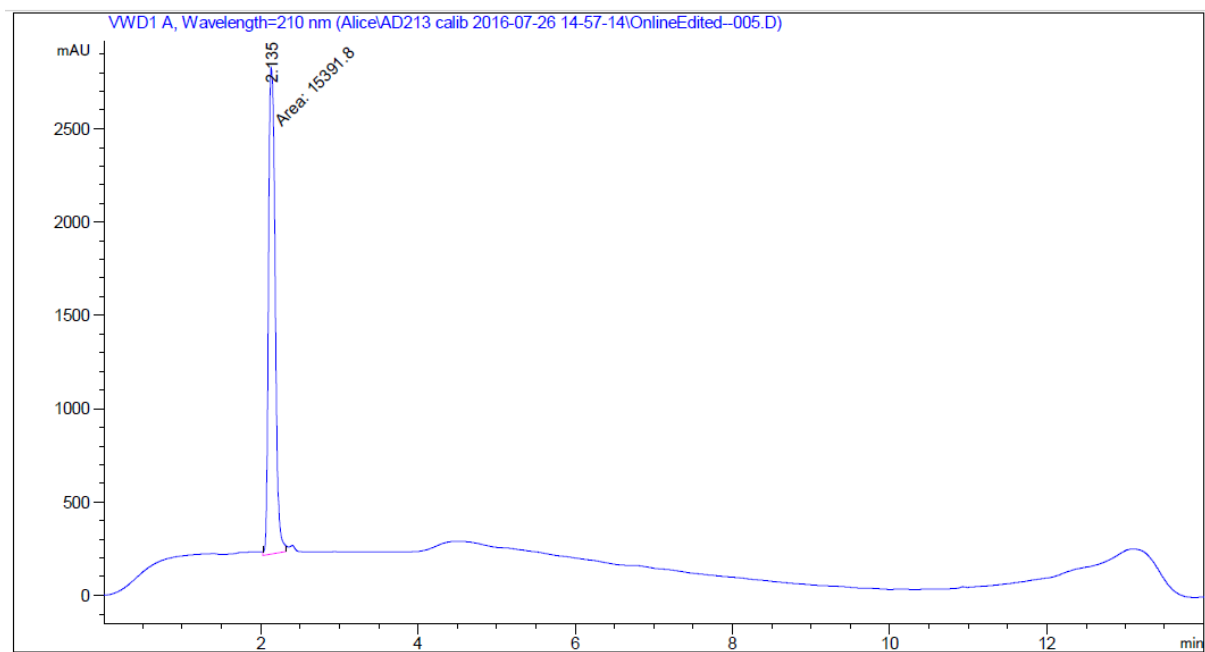
5-Hydroxymethylfurfurylamine **2b** (HPLC Method B)



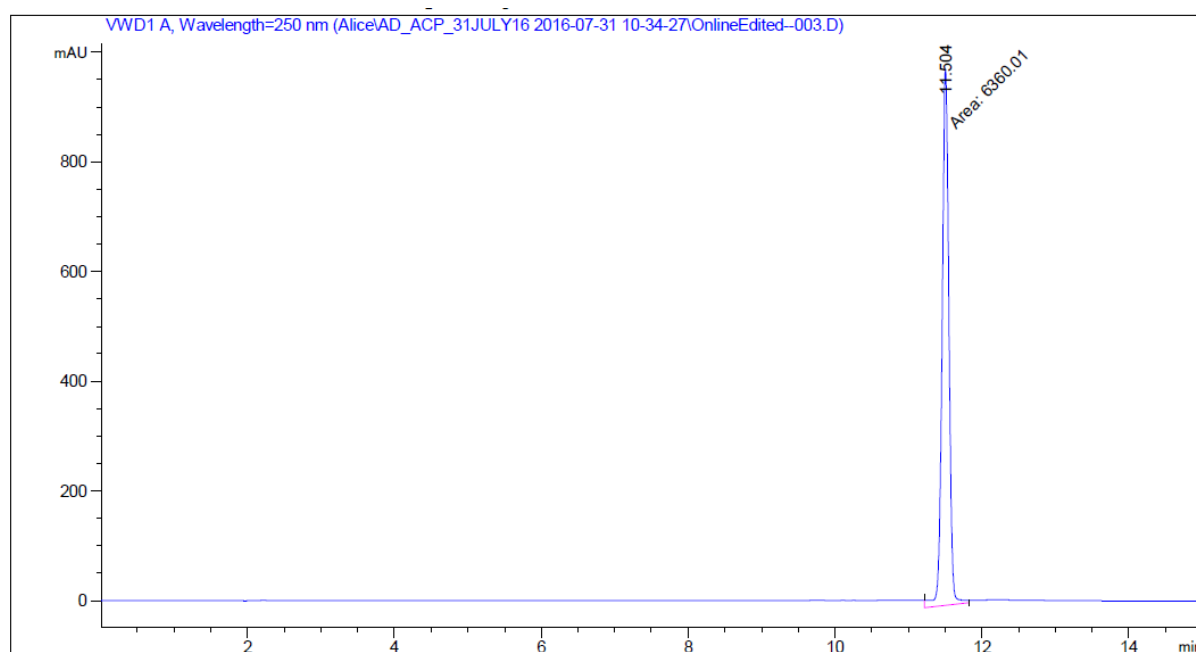
5-Aminomethyl-2-furancarboxylic acid **8b** (HPLC Method A)



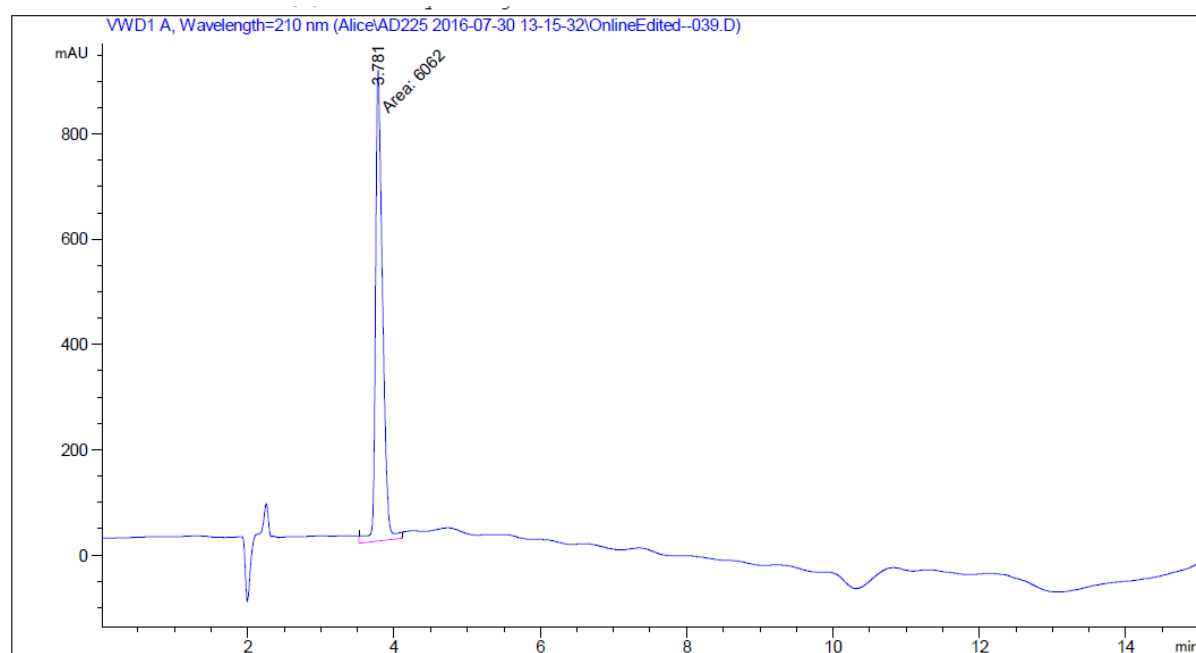
2,5-Bisaminomethylfuran **9b** (HPLC Method C)



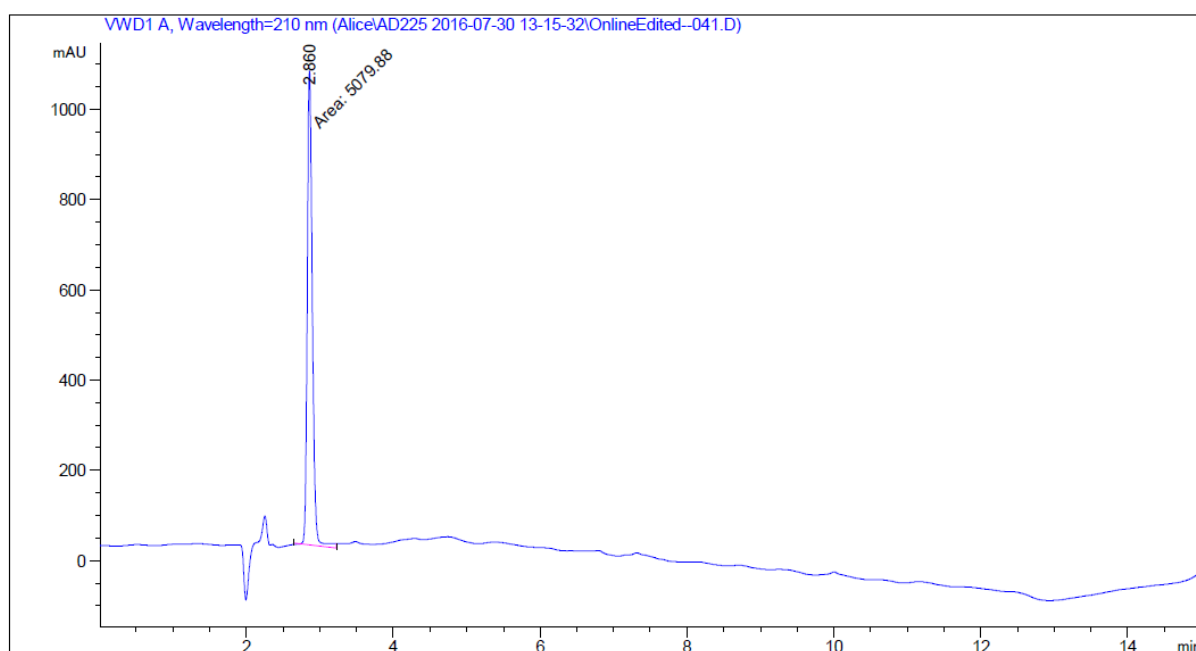
Acetophenone (HPLC Method A)



Benzylamine (HPLC Method B)



Furan-2-ethylamine **10b** (HPLC Method B)



SDS-PAGE Gel of TAmS

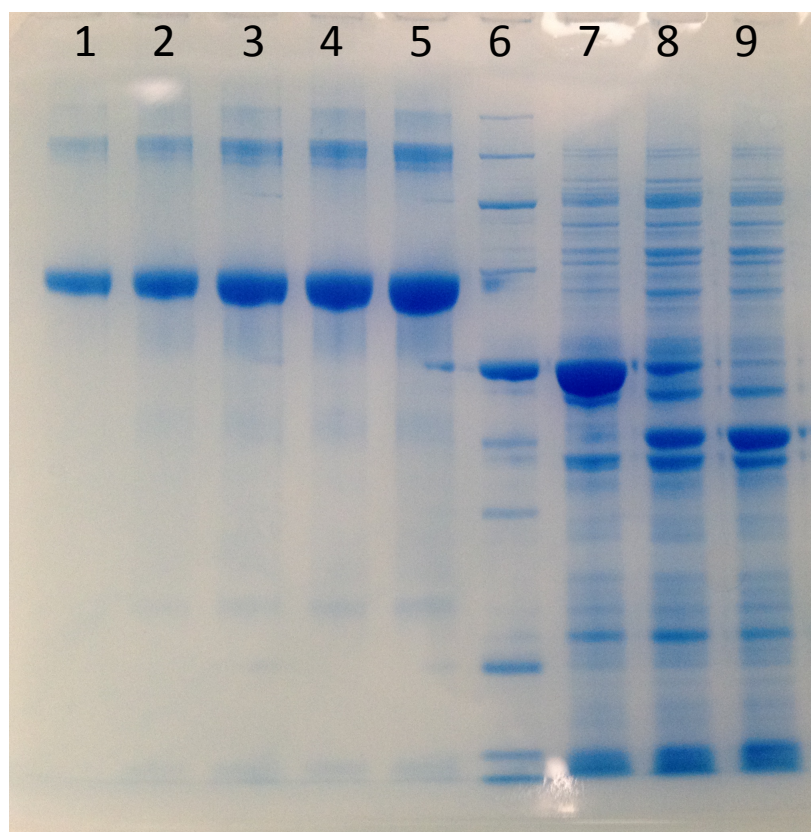
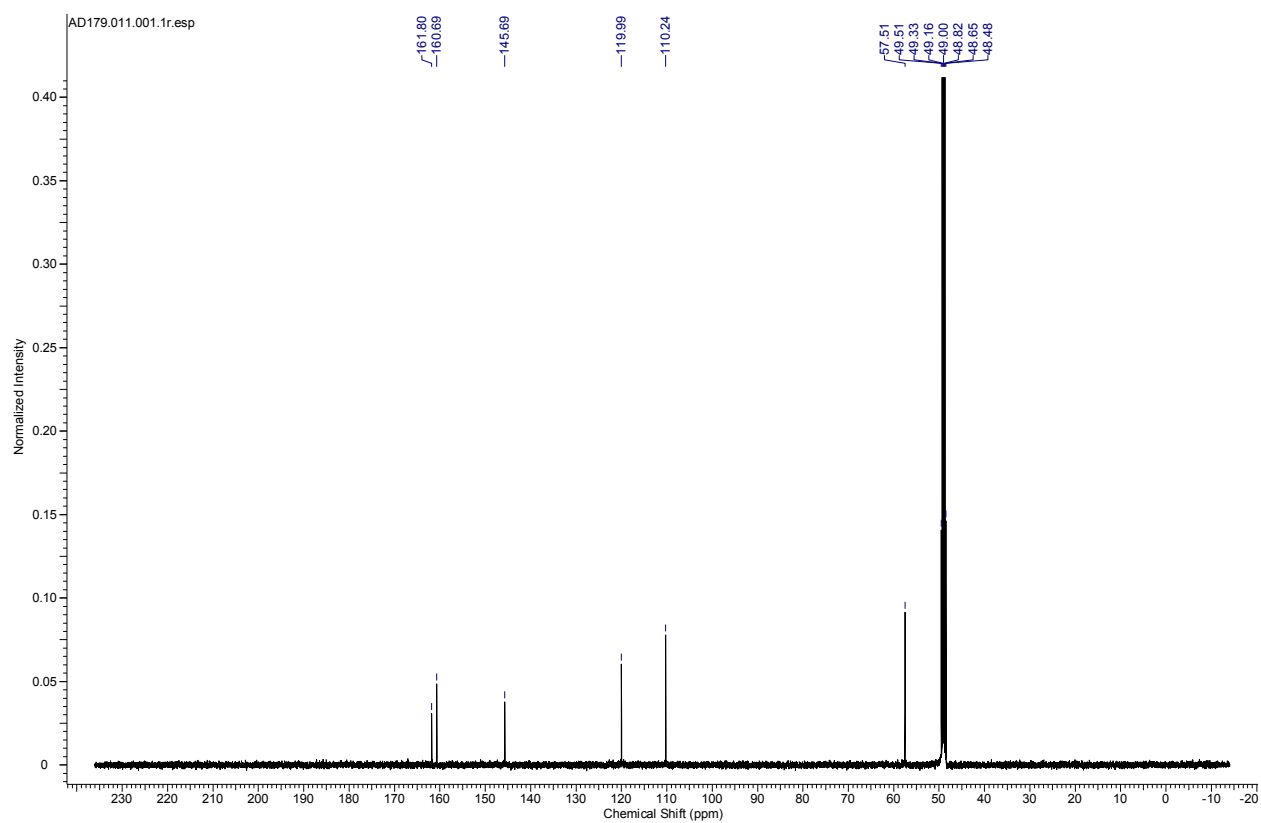
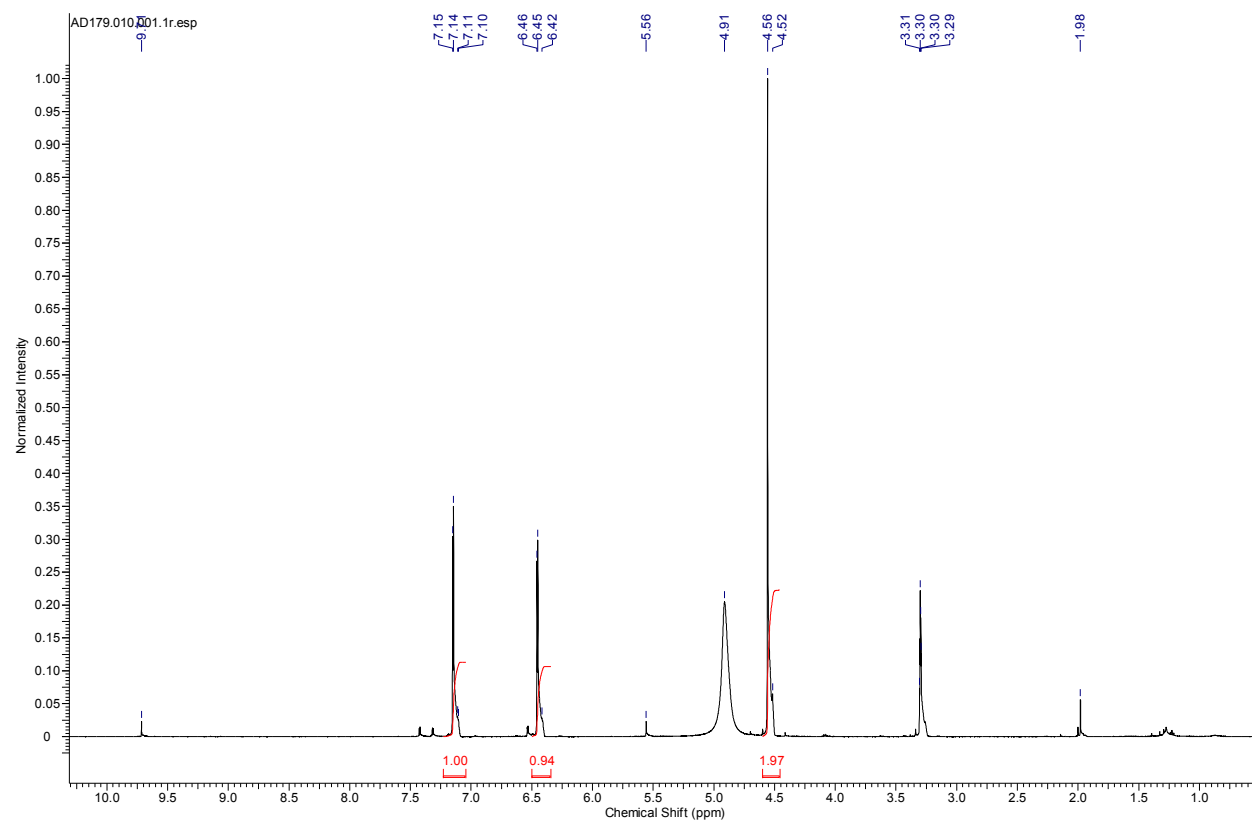


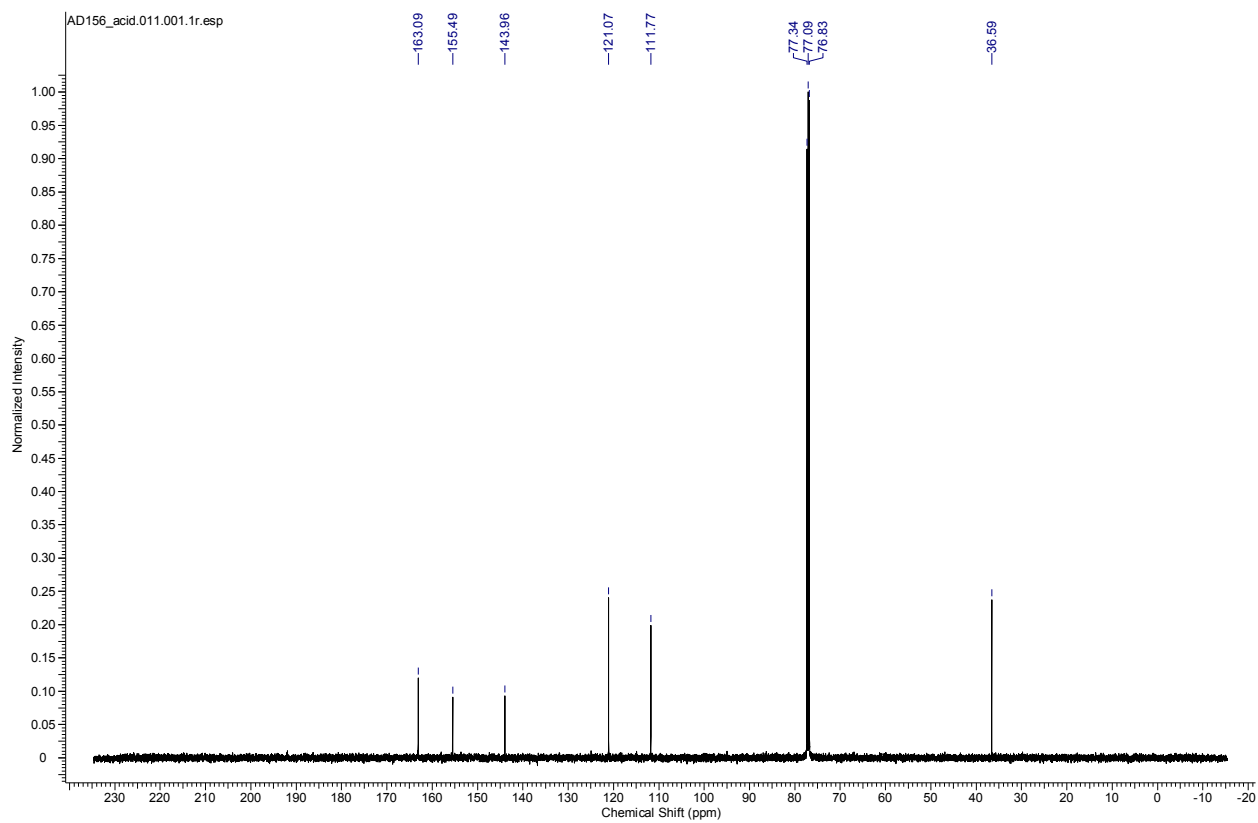
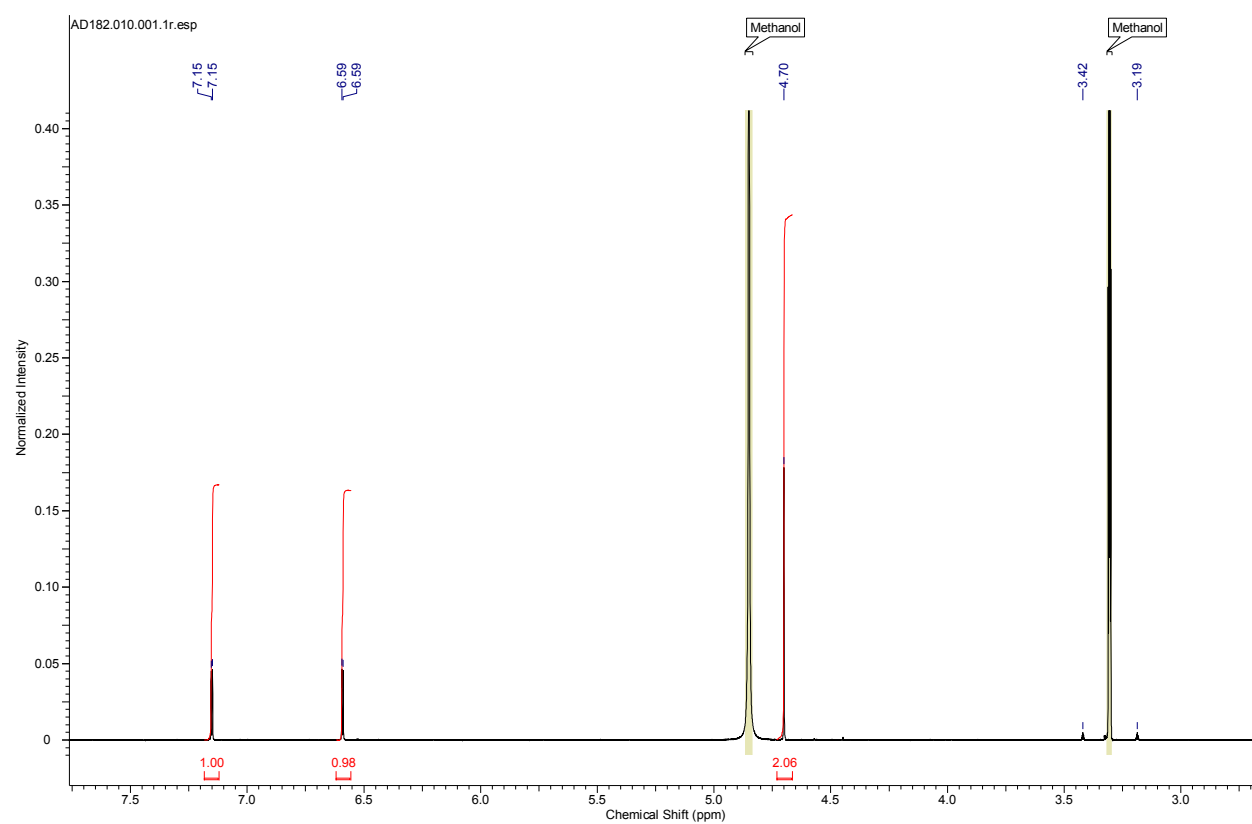
Figure 1. SDS-PAGE gel of induced TAmS and increasing amounts of BSA standard (Sigma Aldrich). **Lanes 1-5:** Increasing amount of BSA (1.0, 1.6, 2.0, 2.4, 3.2, 3.6 μ g). **Lane 6:** Protein markers (Thermo Scientific, 5-250 kDa). **Lanes 7-9:** CV-TAm, Mv-TAm and ArRMut11 respectively (10 μ g total protein).

NMR Spectra

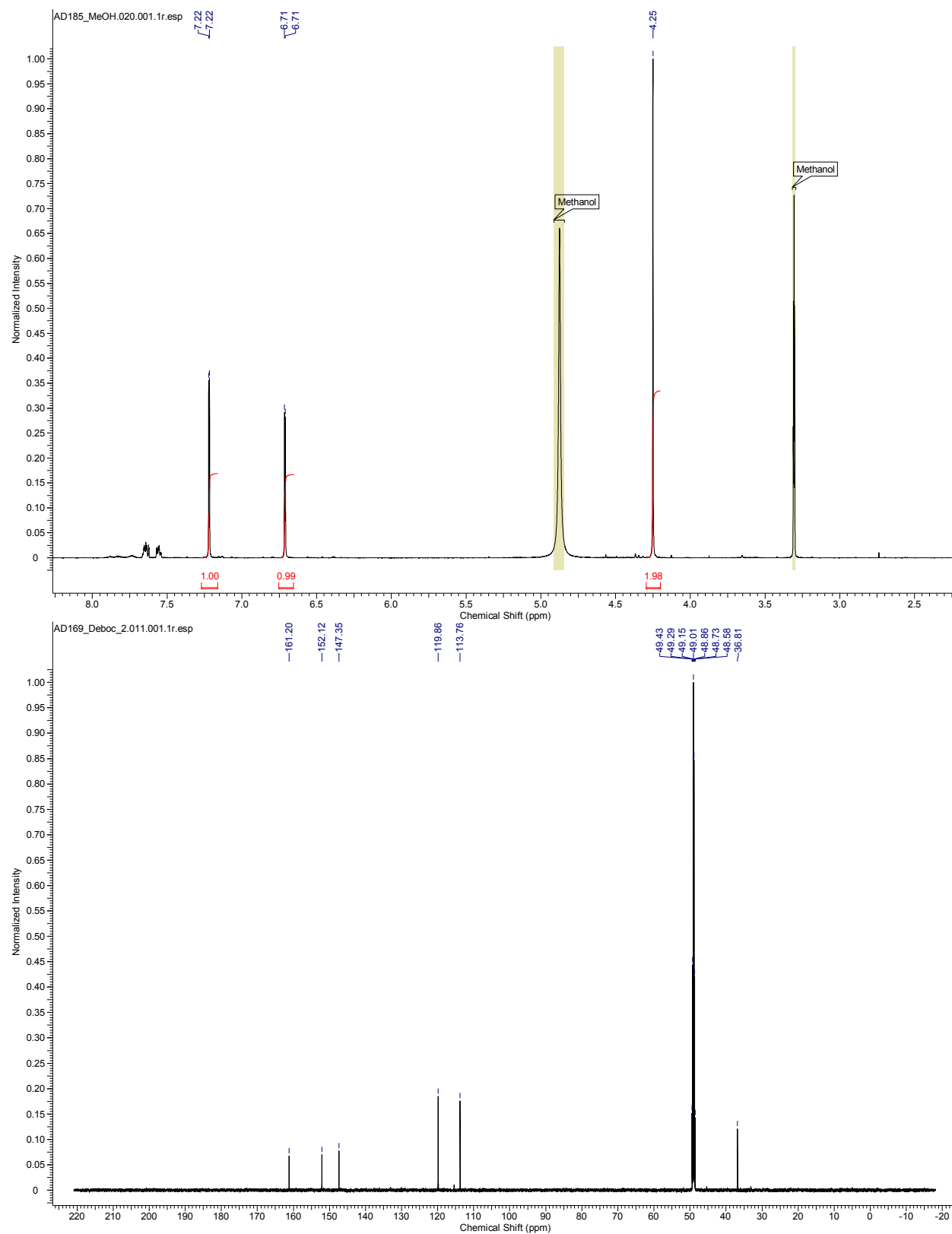
5-Hydroxymethyl-2-furancarboxylic acid



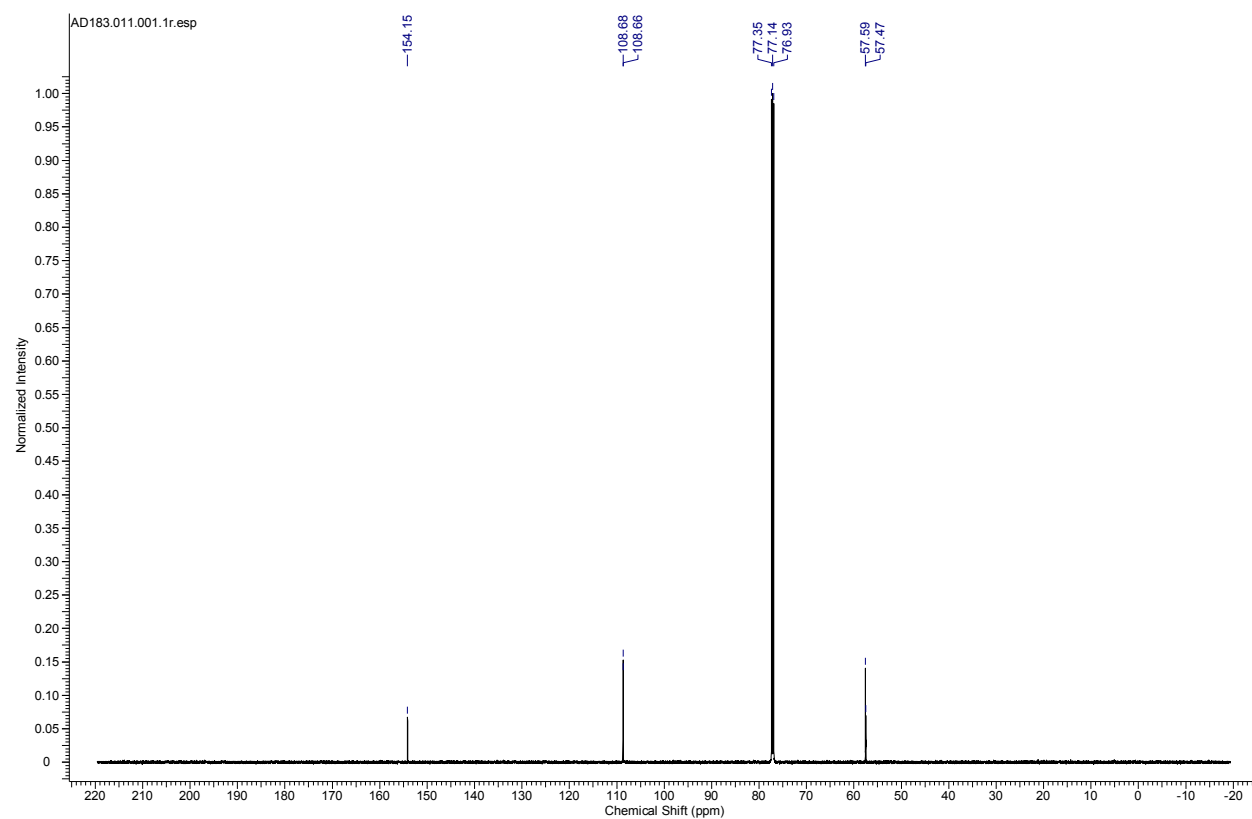
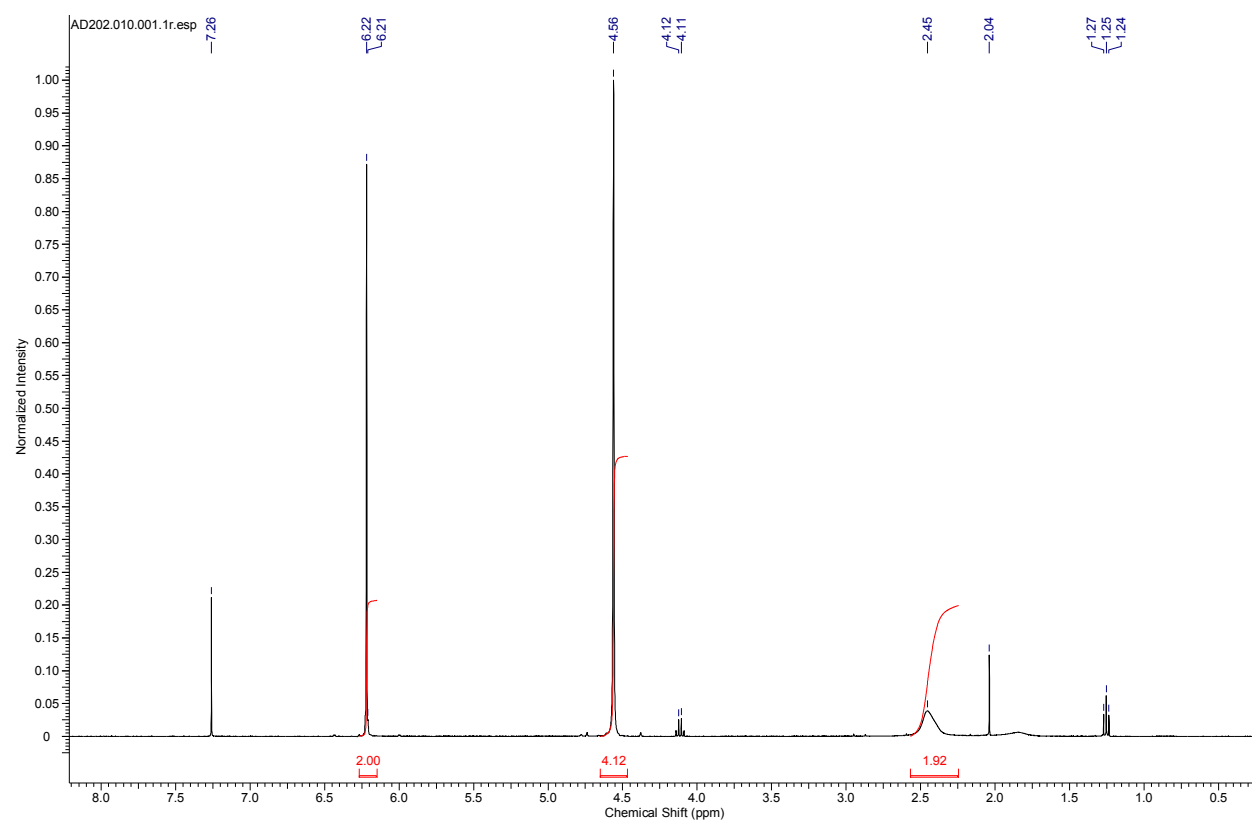
5-Chloromethyl-2-furancarboxylic acid **15**



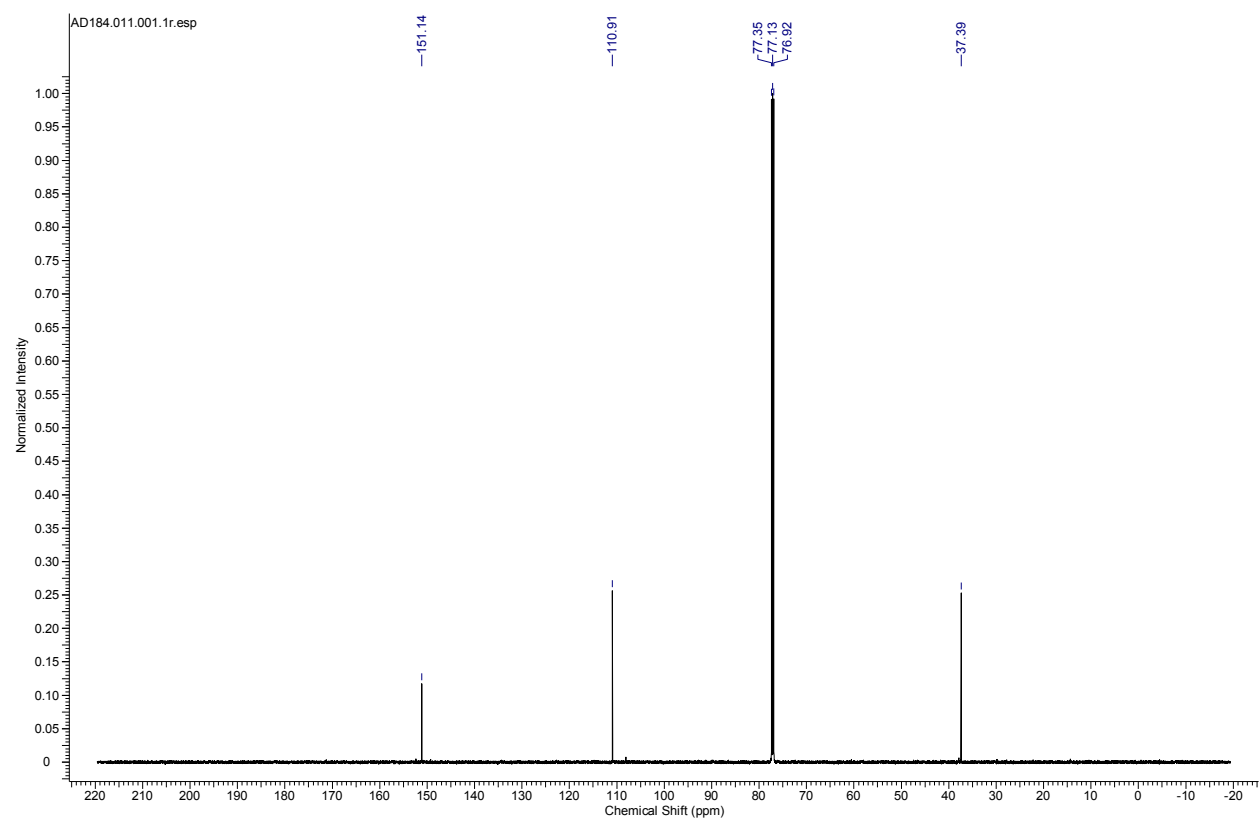
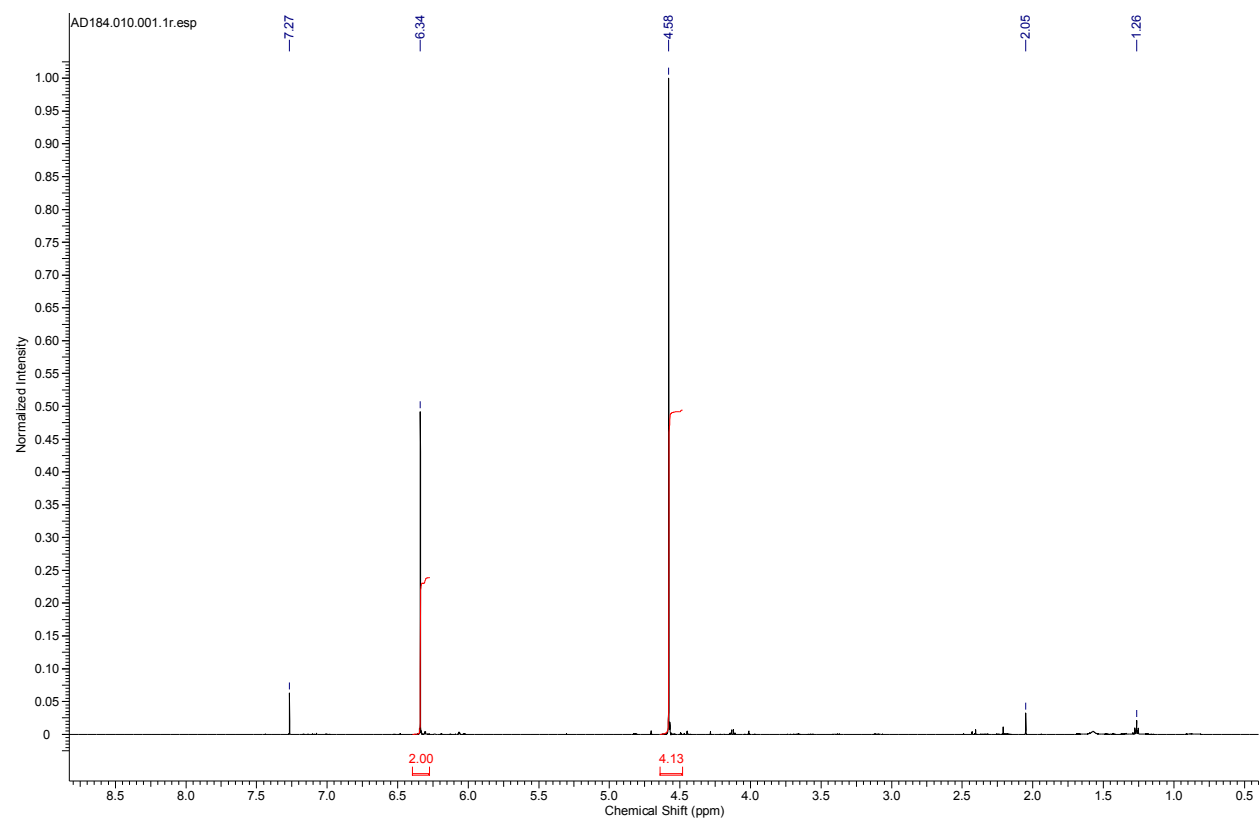
5-Aminomethyl-2-furancarboxylic acid **8b**



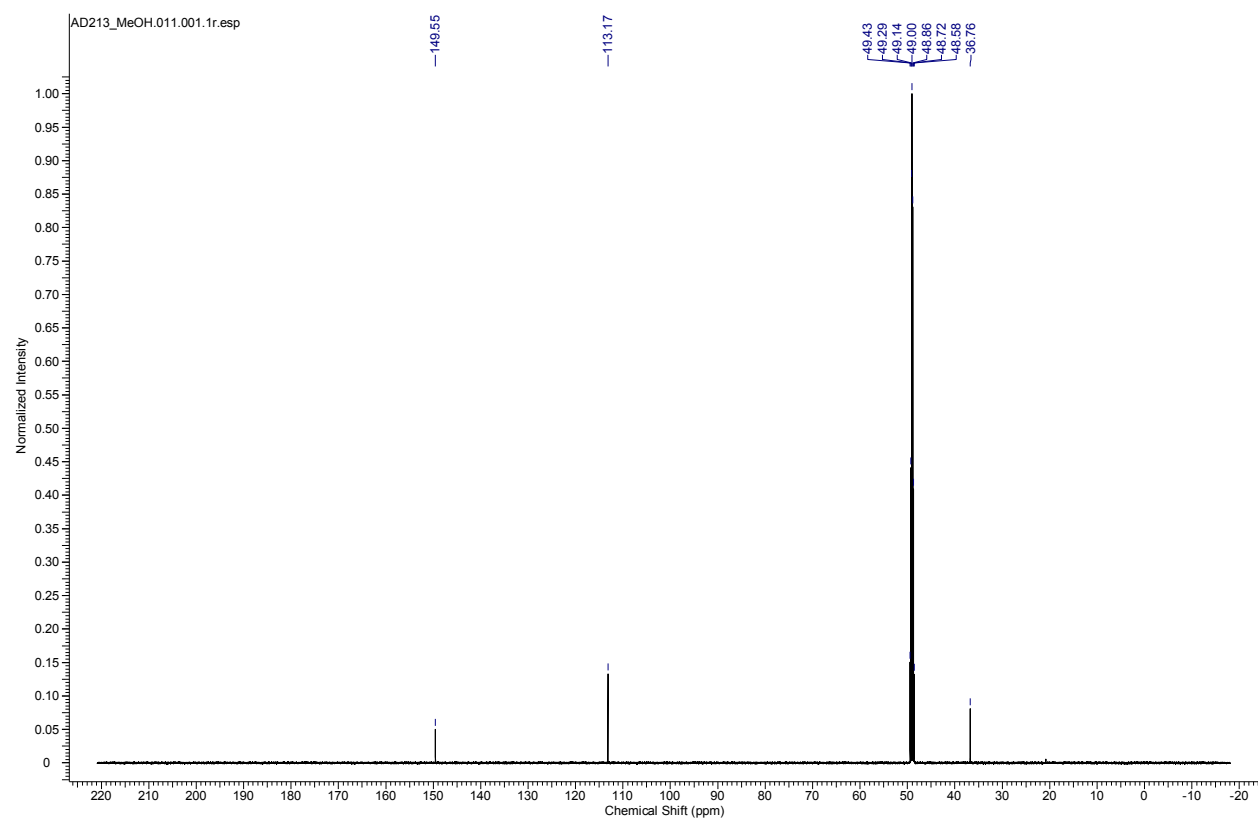
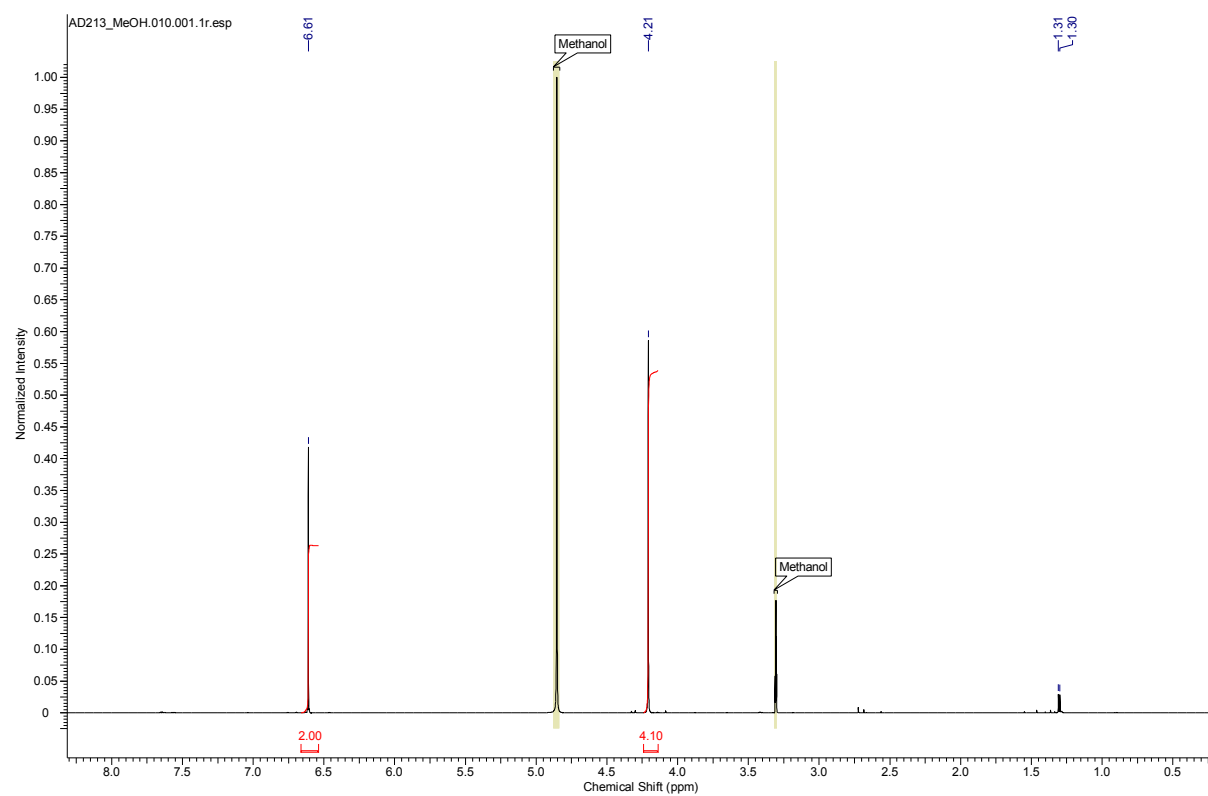
2,5-Bis(hydroxymethyl)furan



2,5-Bis(chloromethyl)furan **14**



2,5-Bis(aminomethyl)furan **9b**



5-Hydroxymethylfurfurylamine **2b**

