Supporting Information

Synthesis of hydrophilic HYNIC-[1,2,4,5]tetrazine conjugates and their use in antibody pretargeting with ^{99m}Tc

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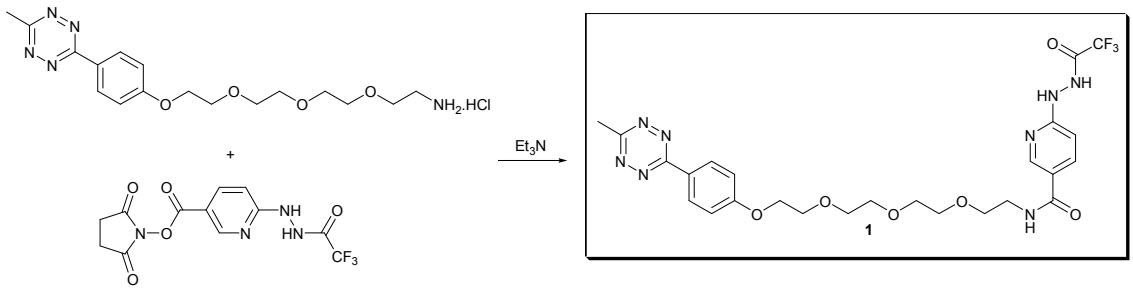
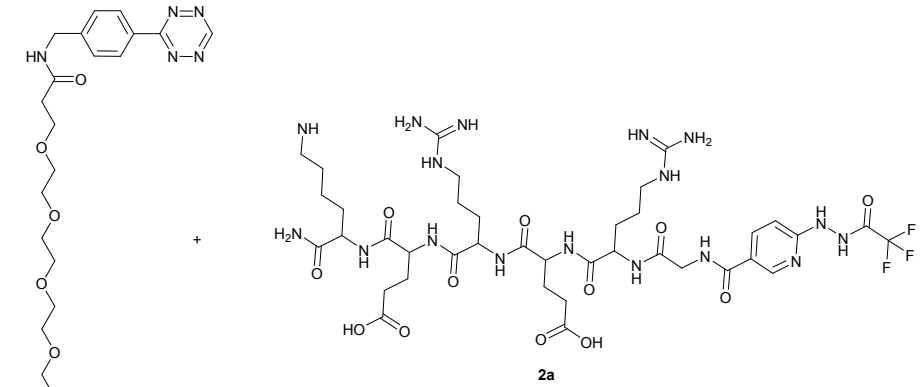
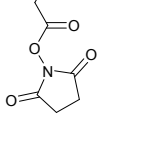
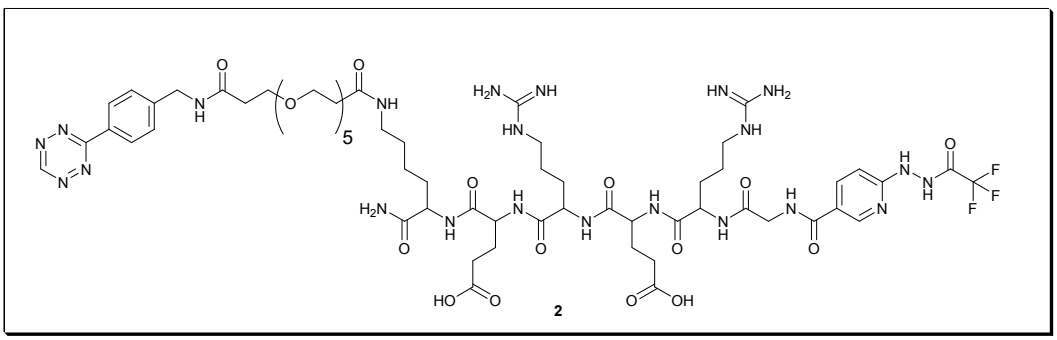
Scheme S1. Synthesis of compound 1 and 2









RP-HPLC control of ^{99m}Tc radiolabeled TZ

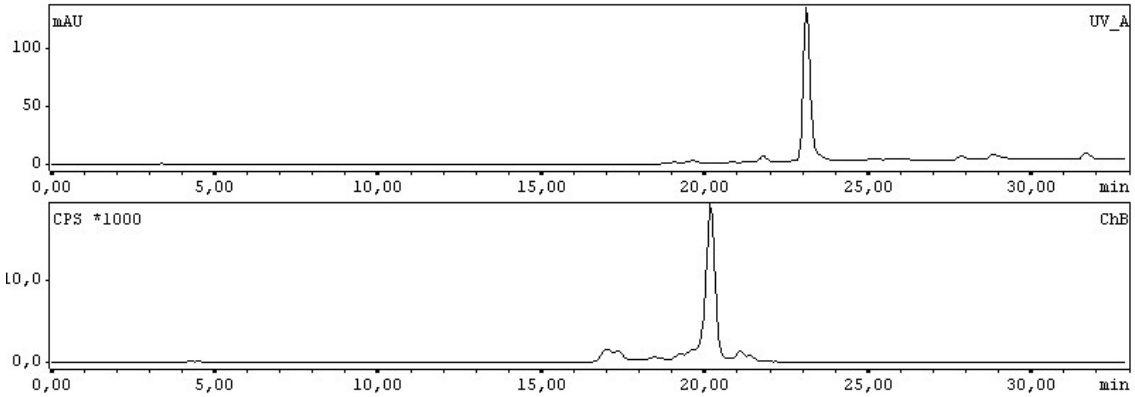


Figure S1: RP-HPLC analysis of ^{99m}Tc-**1**. UV profile (top) and radiochromatogram profile (bottom).

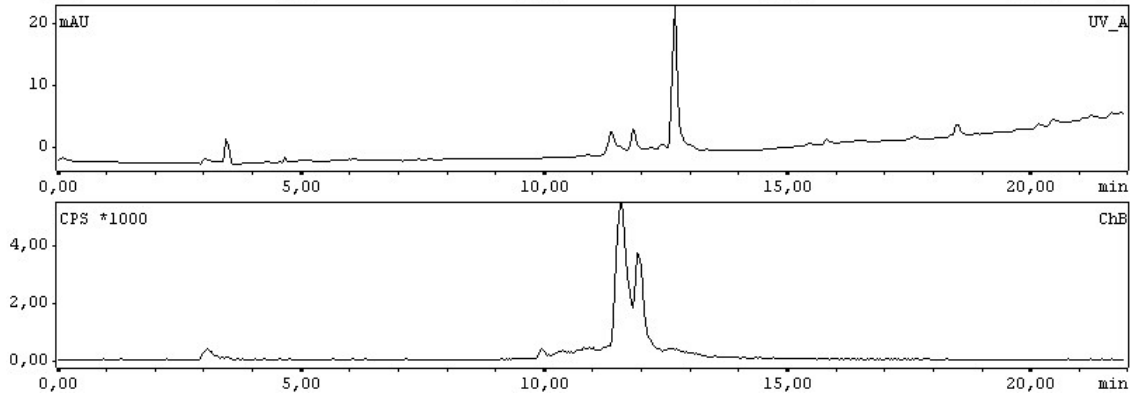


Figure S2: RP-HPLC analysis of ^{99m}Tc-**2**. UV profile (top) and radiochromatogram profile (bottom).

Reaction Kinetics

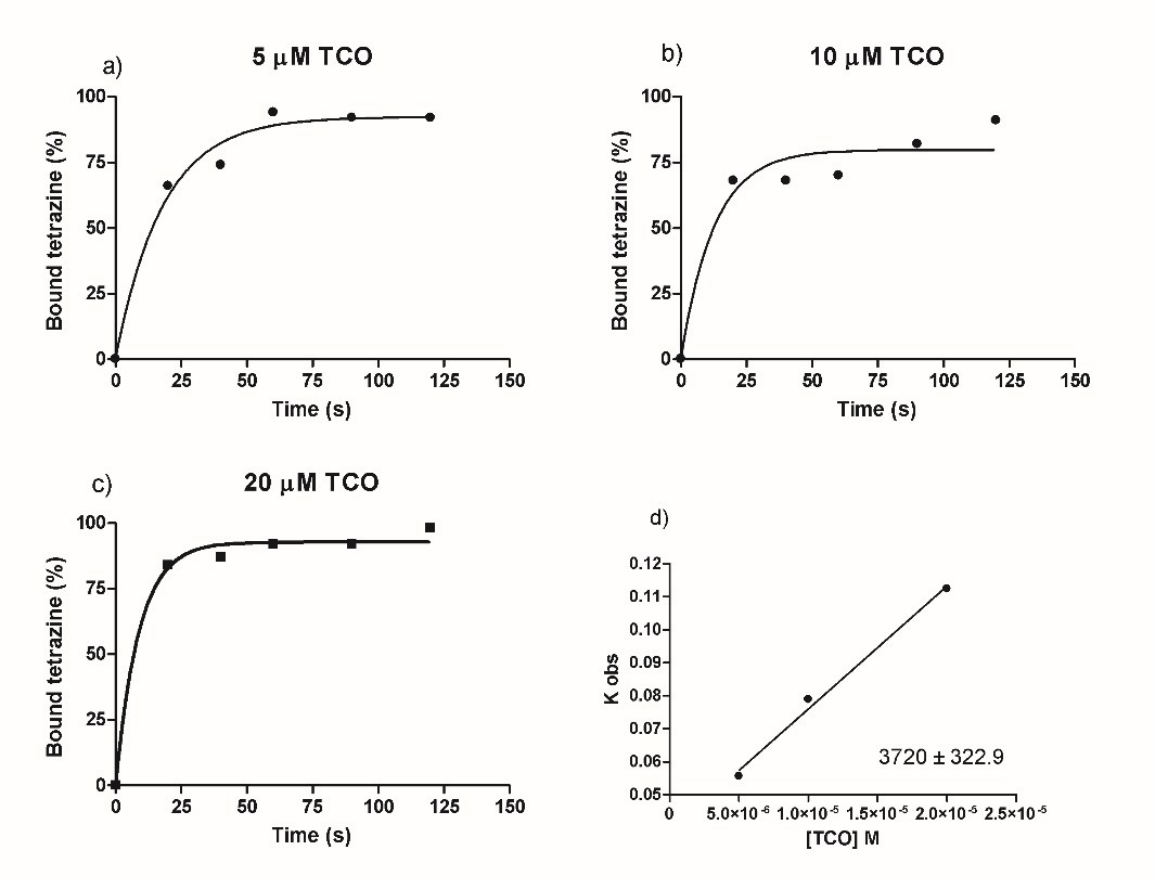


Figure S3: Kinetic studies of the reaction between (*E*)-cyclooctene-system (TCO) and ^{99m}Tc-**2** in PBS at 25 °C.

^{18}FDG PET imaging

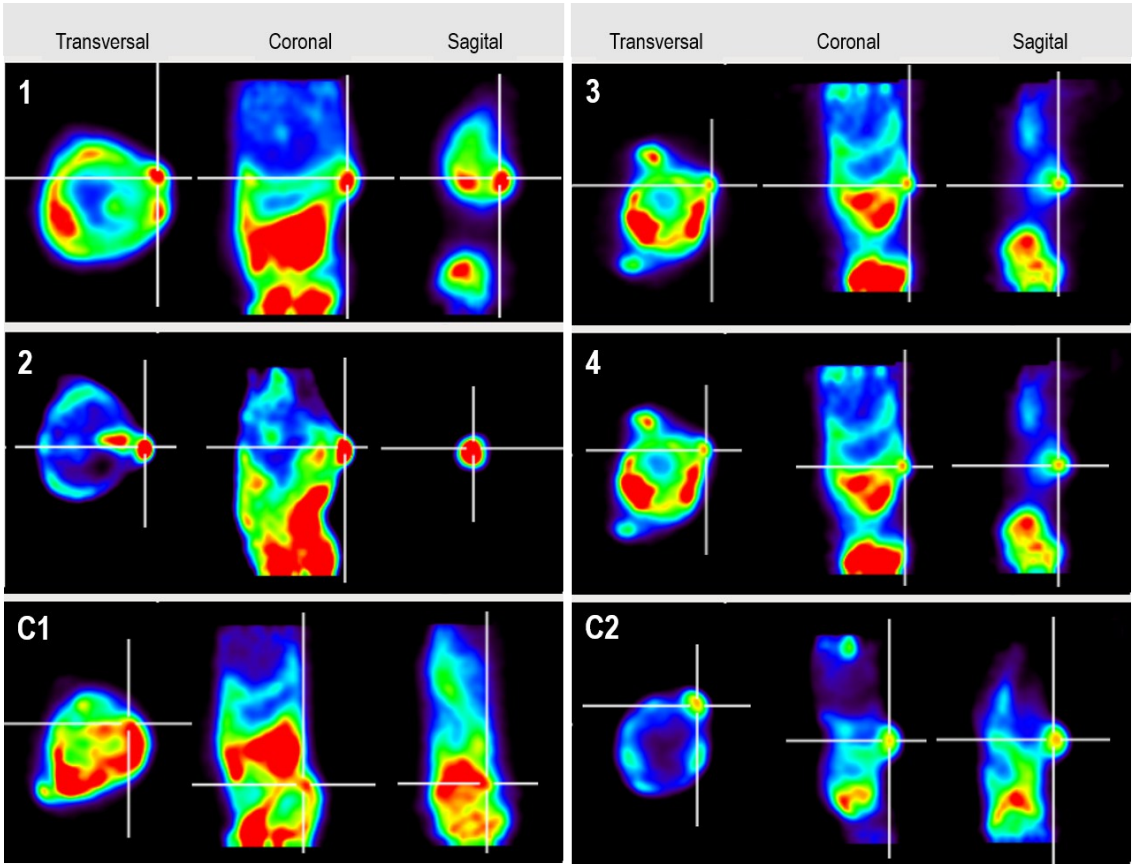


Figure S4: ^{18}FDG PET imaging in B16F10 melanoma tumour bearing mice. White cross indicates the tumour location.

^{99m}Tc SPECT-CT pretargeted imaging

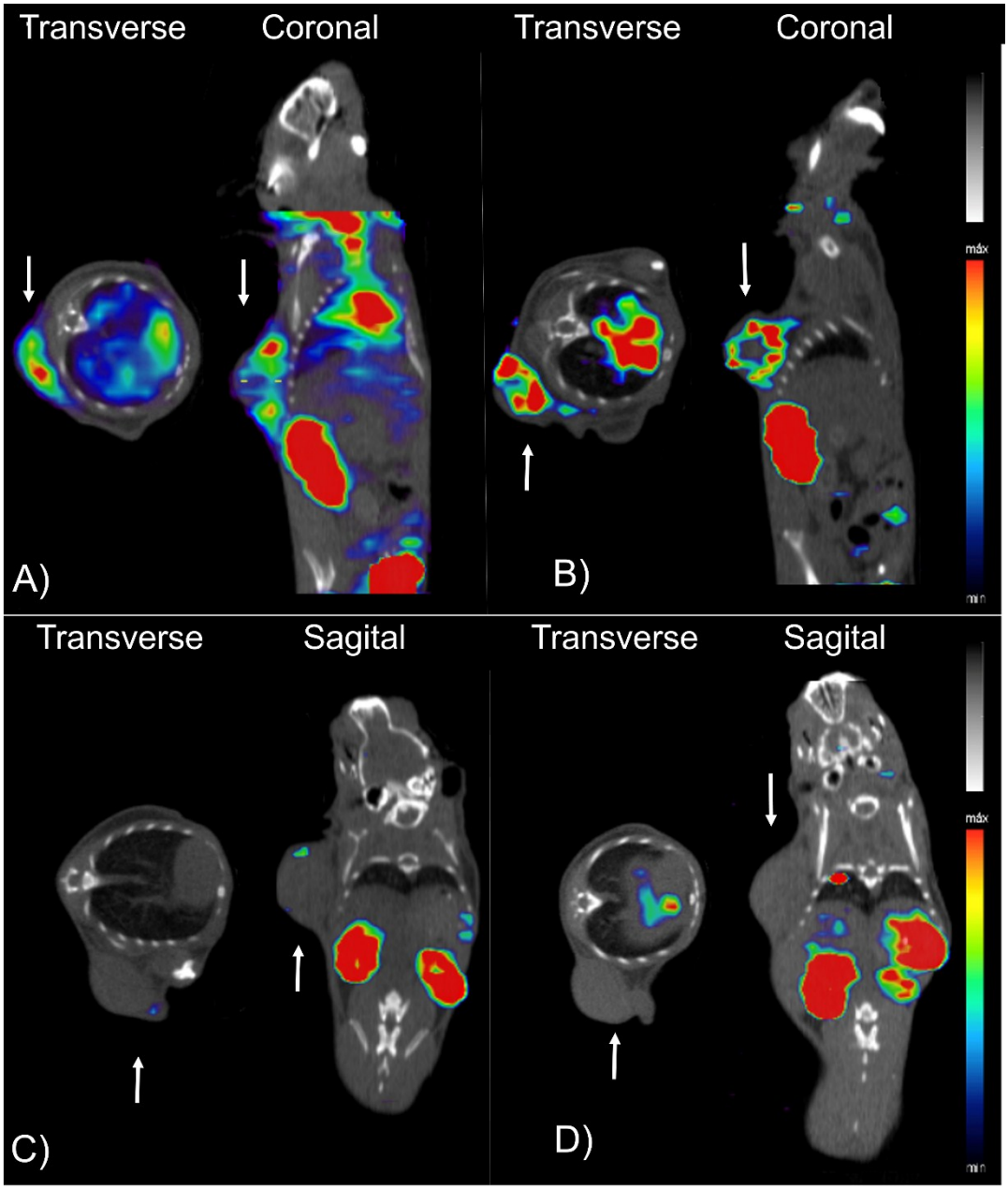


Figure S5: Pretargeted VEGF tumour imaging. SPECT-CT imaging A) 1 h and B) 4 h post ^{99m}Tc-**2** administration in mice pretargeted with bevacizumab-TCO. Control SPECT-CT imaging C) 1 h and D) 4 h post ^{99m}Tc-**2** administration in mice pretargeted with non-conjugated bevacizumab. White arrow indicates tumor location.

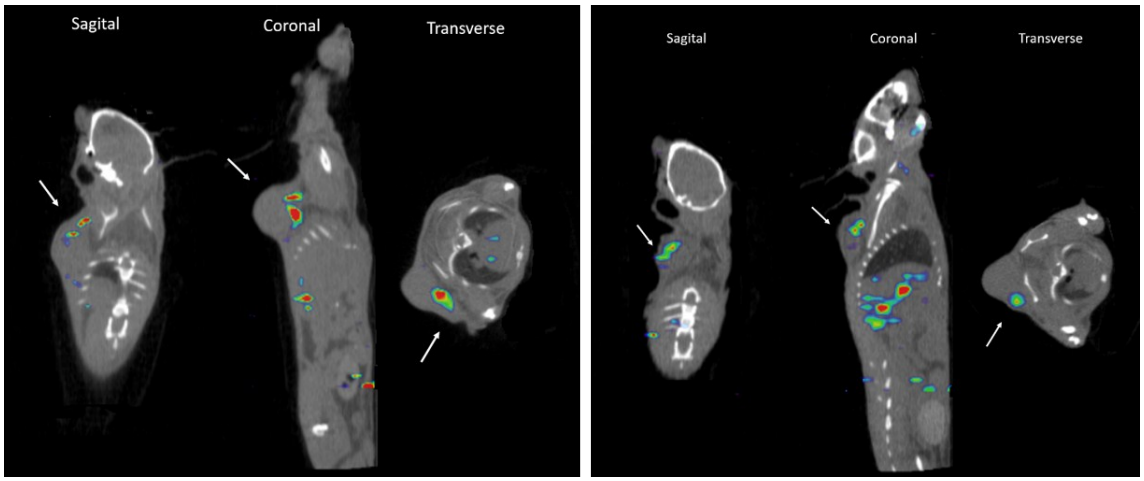


Figure S6: Examples of tumour uptake after 24 h of ^{99m}Tc-**2** for two mice. White arrow indicates tumour location.

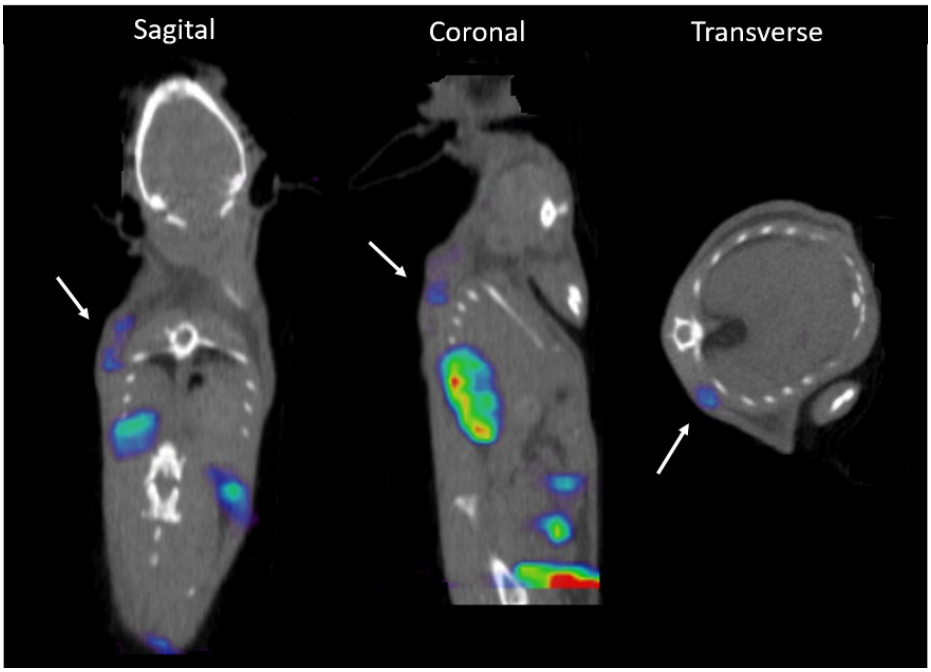


Figure S7: SPECT imaging in control mice blocked with 5 mg of bevacizumab after 1 h of injection of ^{99m}Tc-**2**. White arrow indicates tumour location.

HPLC (UV detection) profile of compound 1

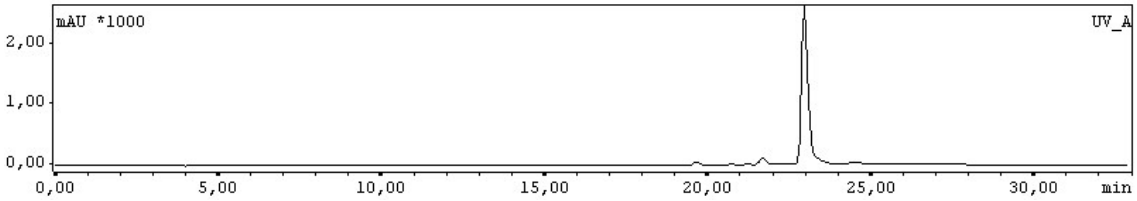


Figure S8: Reverse phase -HPLC analysis of derivative **1** (detection UV 280 nm).

NMR Spectroscopy of compound 1

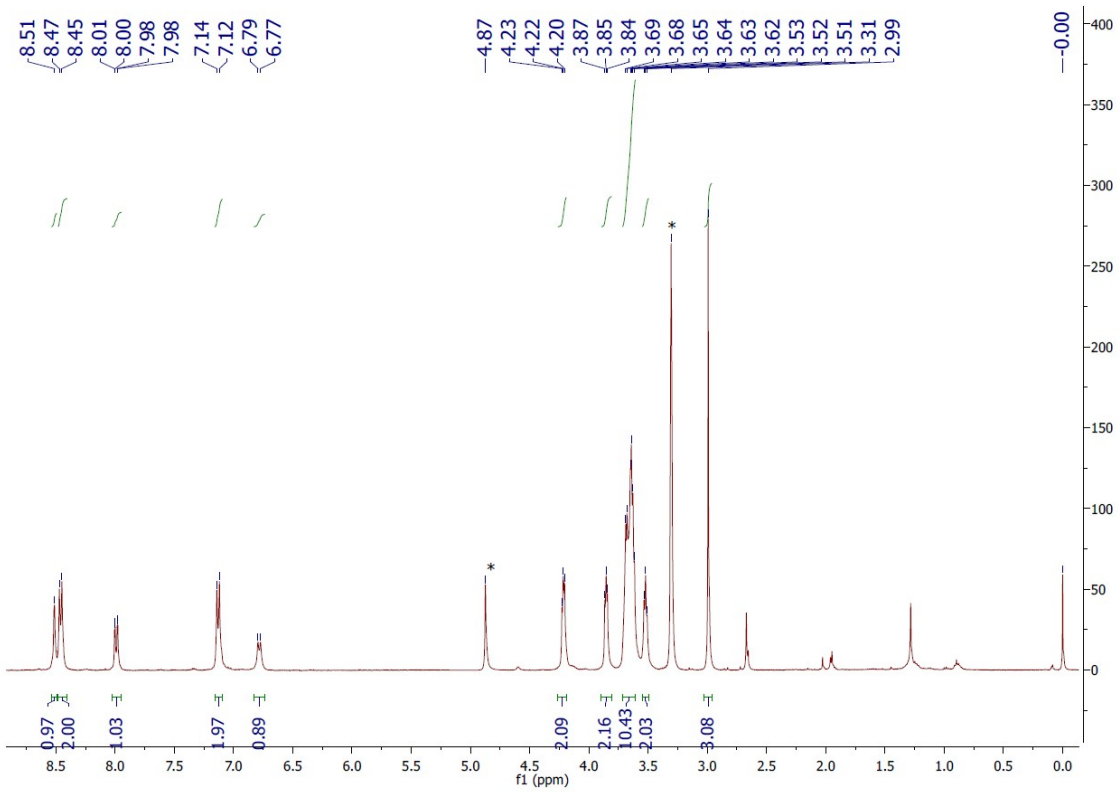


Figure S9: ^{1}H-NMR spectrum of derivative **1** (400 MHz, 303 K, MeOH-d_4:D_2O). *MeOH and water (residual solvent peaks).

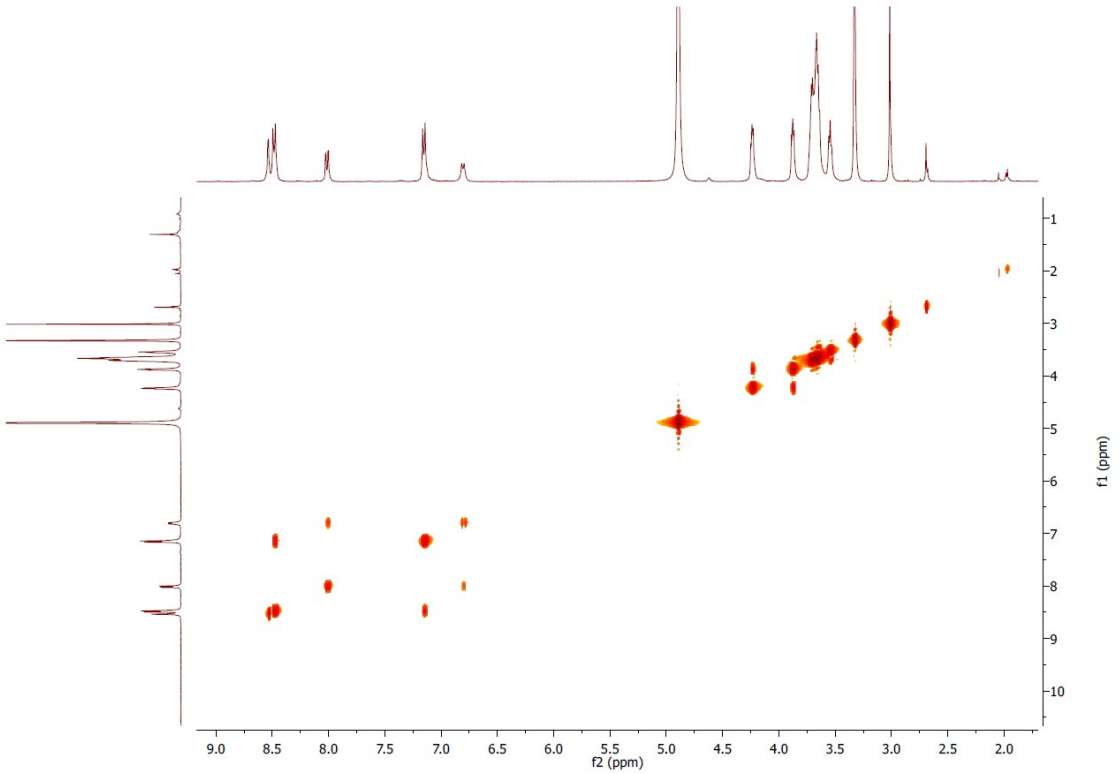


Figure S10: ^{1}H-^{1}H COSY NMR experiment of derivative **1**.

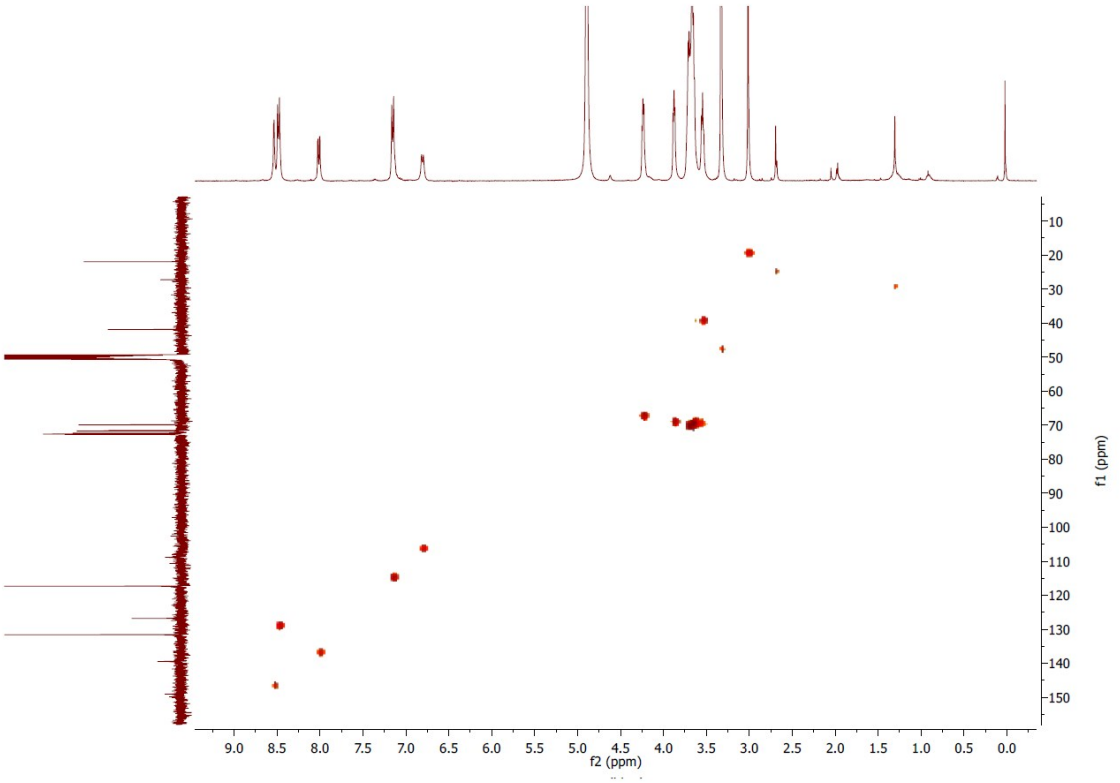


Figure S11: ^{1}H-^{13}C HSQC NMR experiment of derivative **1**.

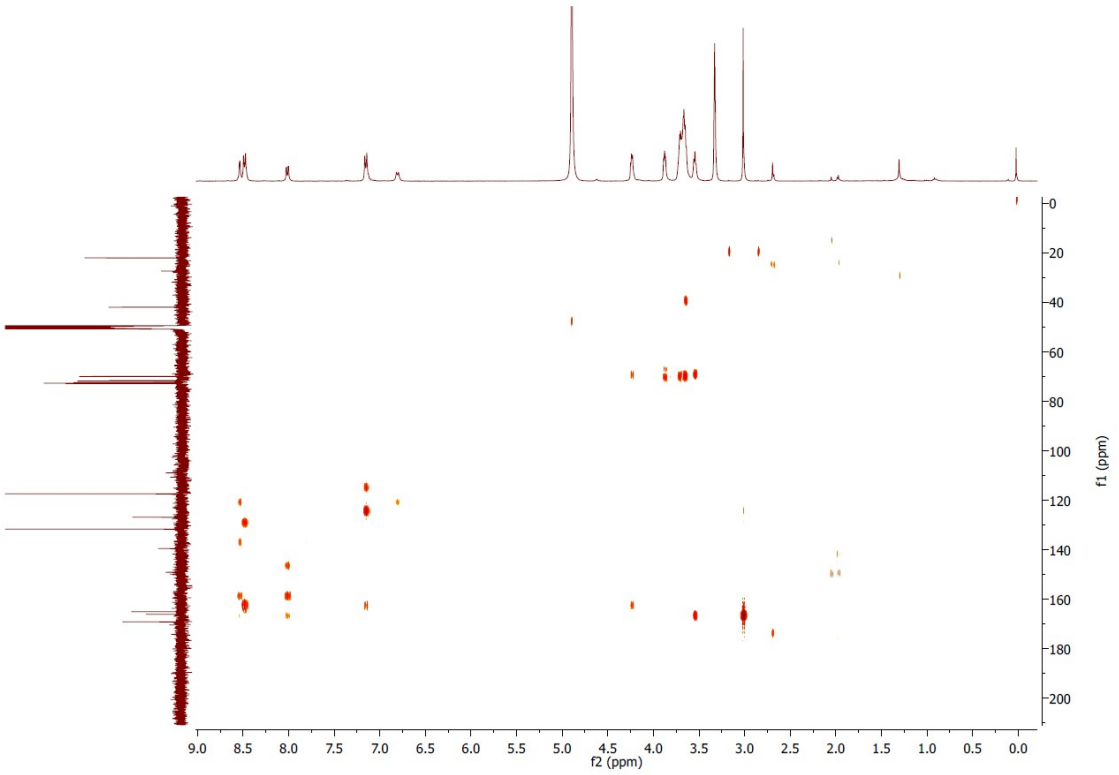


Figure S12: ^{1}H-^{13}C HSBC NMR experiment of derivative **1**.

Mass Spectrometry of compound 1

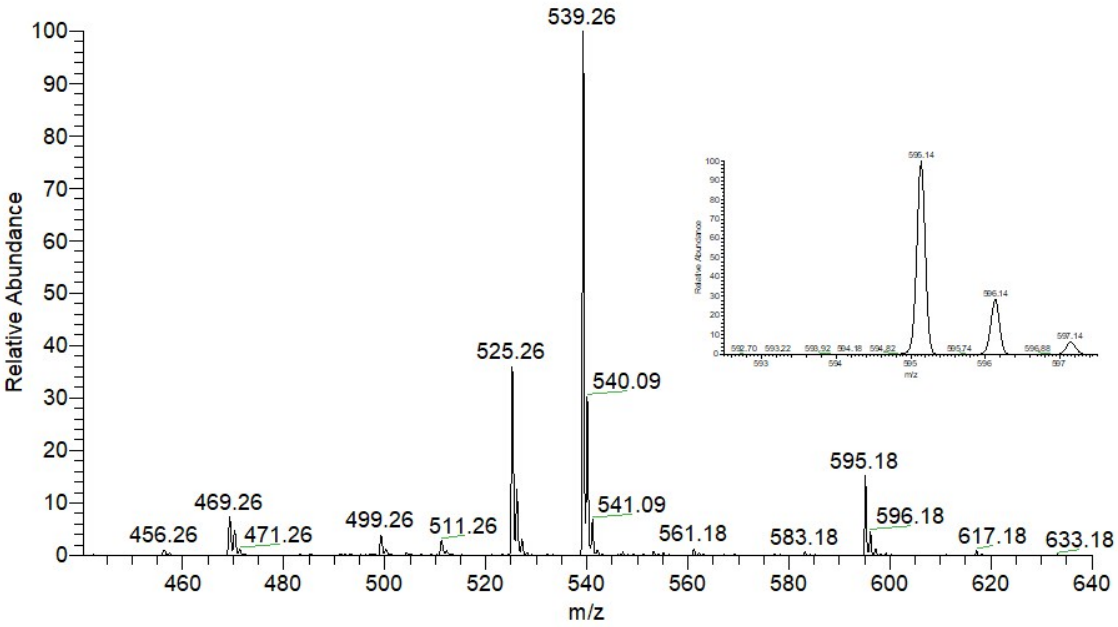


Figure S 13: Full MS scan of derivative **1**.

HPLC (UV detection) profile of compound 2

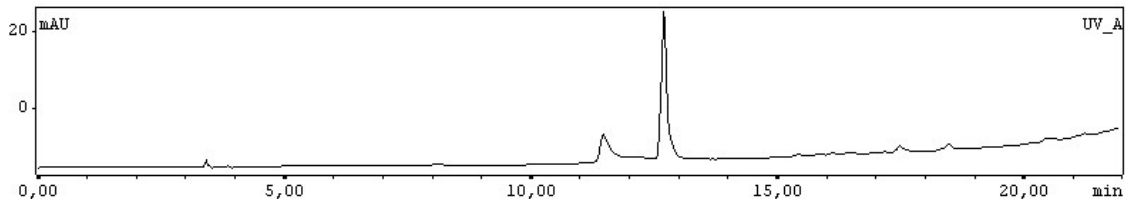


Figure S14: Reverse phase -HPLC analysis of derivative **2** (UV detection, 280 nm).

Mass Spectrometry of compound 2

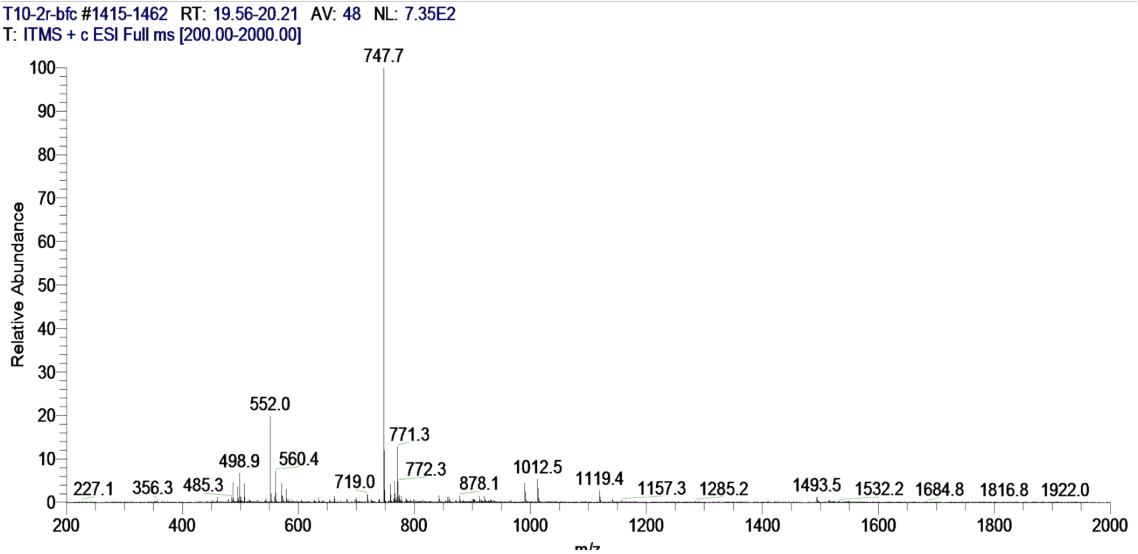


Figure S15: Full MS scan of derivative **2**.