Supplementary Materials: Synthesis and Evaluation of ^{99m}Tc-Tricabonyl Labeled Isonitrile Conjugates for Prostate-Specific Membrane Antigen (PSMA) Image

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RP-HPLC purification non-radioactive compounds

RP-HPLC was performed with a Gilson, equipped with a 506C system interface, a 155 UV/VIS detector (dual wavelength 220, 254 nm) and 321 pumps. The operation of Gilson HPLC system is controlled by Trilution software. Purification intermediate and final compounds were carried out by semi-preparative XTerra RP18 10 μm (10 mm × 250 mm) column (Waters Co., U.S.A). The mobile phase consist of 0.1%TFA/water (solvent A), acetonitrile (solvent B) with a gradient method, 0–40 min, 0–100 % B at flow rate 5 mL/min (**method1**) or 0–5 min, 0% B; 5–30 min, 0–100% B at flow rate of 3 mL/min (**method 2**) or 0–35 min, 0–100% B at flow rate 5 mL/min (**method 3**) or 0–40 min, 0–100%B at flow rate 5 mL/min (**method 4**) or water (solvent A), acetonitrile (solvent B) with a gradient method of 0–5 min, 0%B; 5–40 min, 0–100%B at a flow rate of 3 mL/min (**method 5**) or 0–30 min, 10–100% B (**method 6**).

Purification and quality control of [^{99m}Tc]Tc-15 and [^{99m}Tc]Tc-16 conjugates

1. RP-HPLC was performed with a Gilson®, equipped with a 506C system interface, a 155 UV-vis detector, and 321 pumps and radioactive detector. Purification of [^{99m}Tc]Tc-**15** and [^{99m}Tc]Tc-**16** was carried out using a semi-preparative XTerra RP18 10 μm (10 mm × 250 mm)

column (Waters Co., U.S.A). The mobile phase consisted of 0.1%TFA/water (solvent A), 0.1%TFA/methanol (solvent B) with a gradient of; 0–20 min, 0–100%B (**method 7**) or 0–20 min,15–100% B; 20–25 min, 100% B (**method 8**) at a flow rate of 5 mL/min.

2. Analytical RP-HPLC was performed on analytical XTerra RP18 10 μm (10 mm × 250 mm) column (Waters Co., U.S.A). The mobile phase consisted of 0.1%TFA/water (solvent A), 0.1%TFA/methanol (solvent B) with a gradient of; 0–20 min, 0–100% B; 20–25 min, 100% B (**method 9**) at a flow rate of 1 mL/min
3. RadioTLC was performed with TLC-SG with solvent system (methanol:HCl, 99 : 1(v/v))

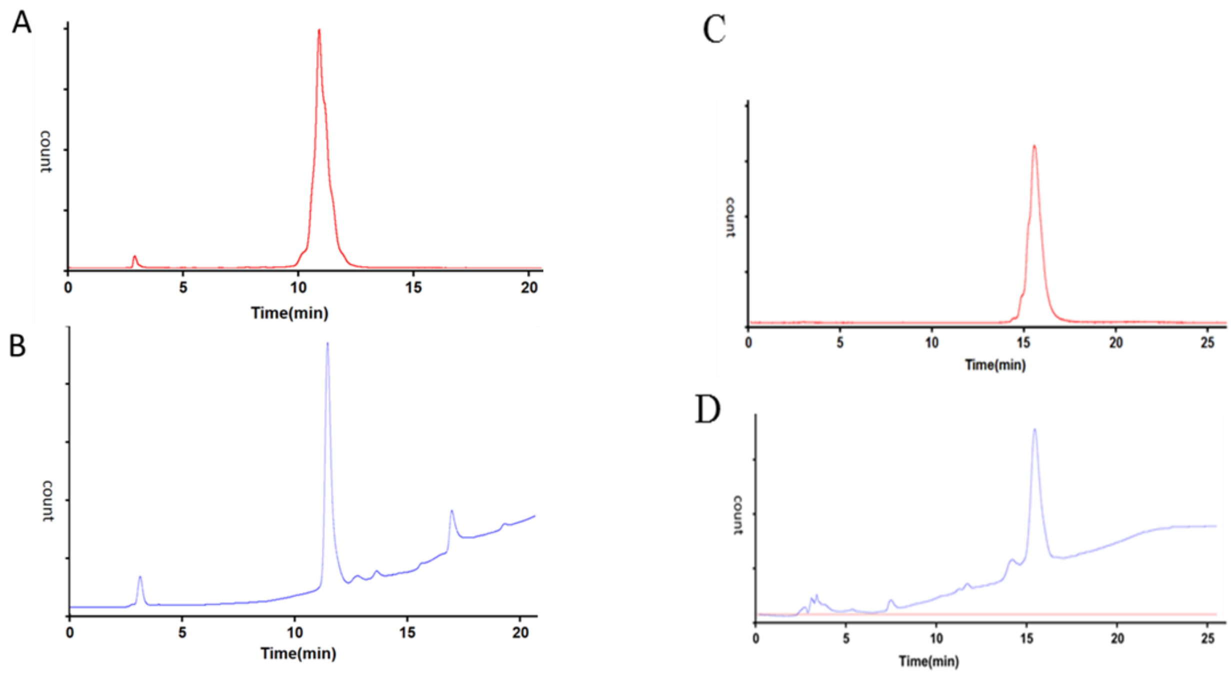

Figure S1: HPLC profiles of hot and cold complexes. (A) [^{99m}Tc]Tc-**15**, (B) Re-**15**, (C) [^{99m}Tc]Tc-**16**, and (D) Re-**16**.

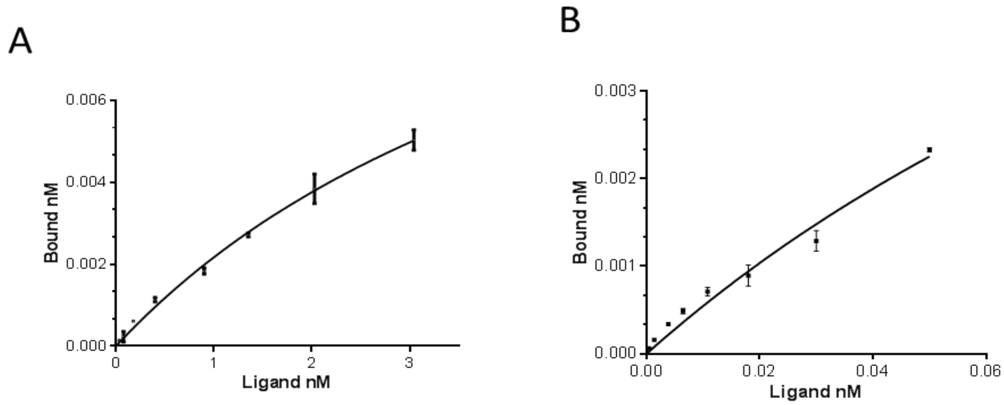

Figure S2: Saturation binding curve (**A**) [^{99m}Tc]Tc-**15** (**B**) [^{99m}Tc]Tc-**16**. The 22Rv1 (1×10^5) cells were incubated at 37 °C for 1 h by increasing concentration of radiotracers. K_d value were determined by non-linear regression

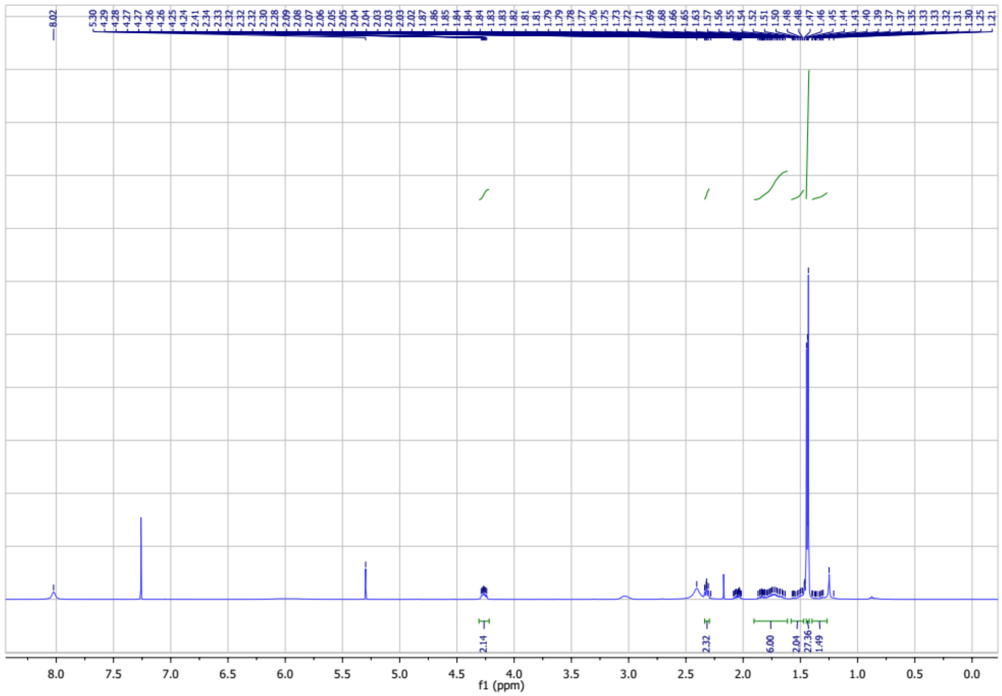

Figure S3. [1]H-NMR of **2**

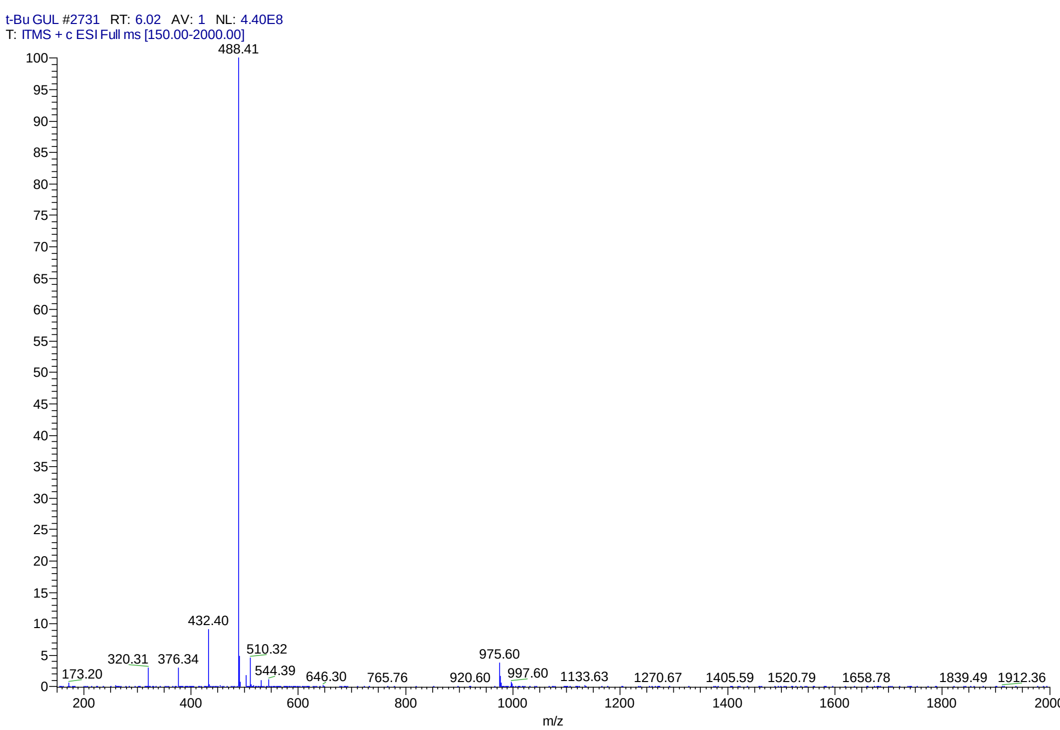

Figure S4: ESI-MS spectrum of **2**

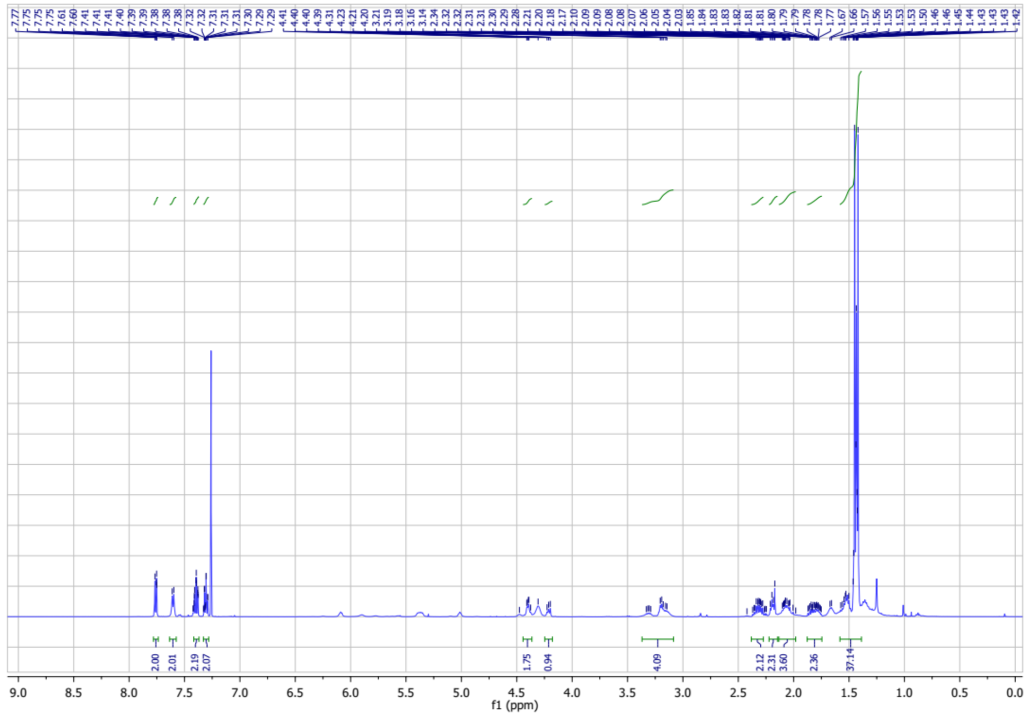

Figure S5. ^{1}H-NMR of **3**

Figure S6: ESI-MS spectrum of **3**

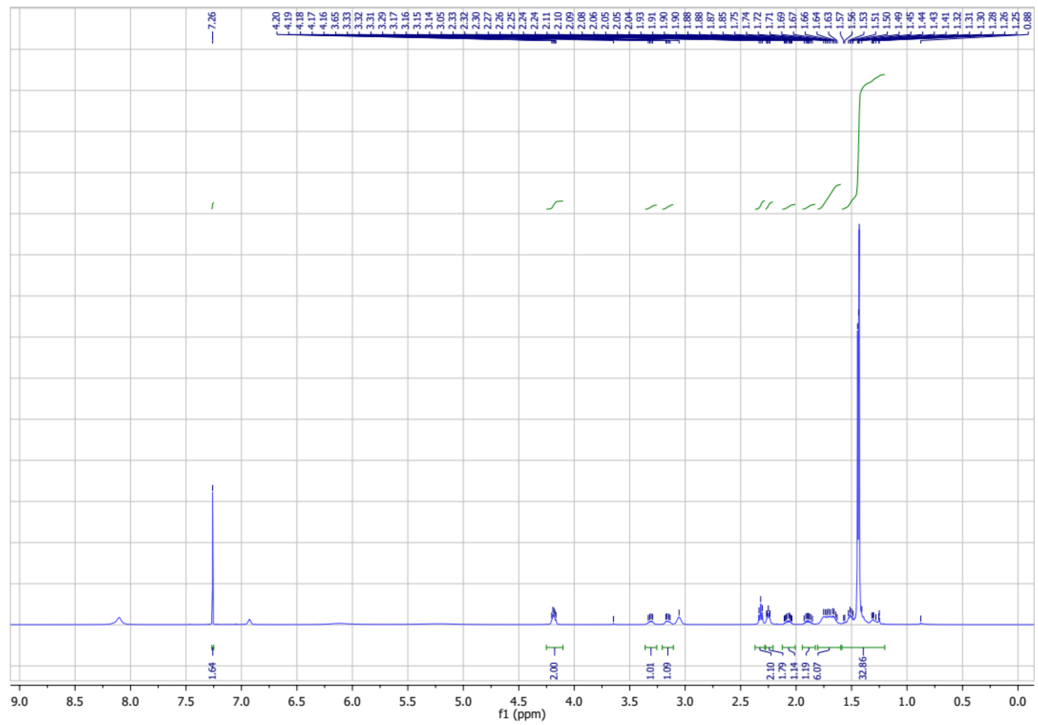

Figure S7. [1]H-NMR of **4**

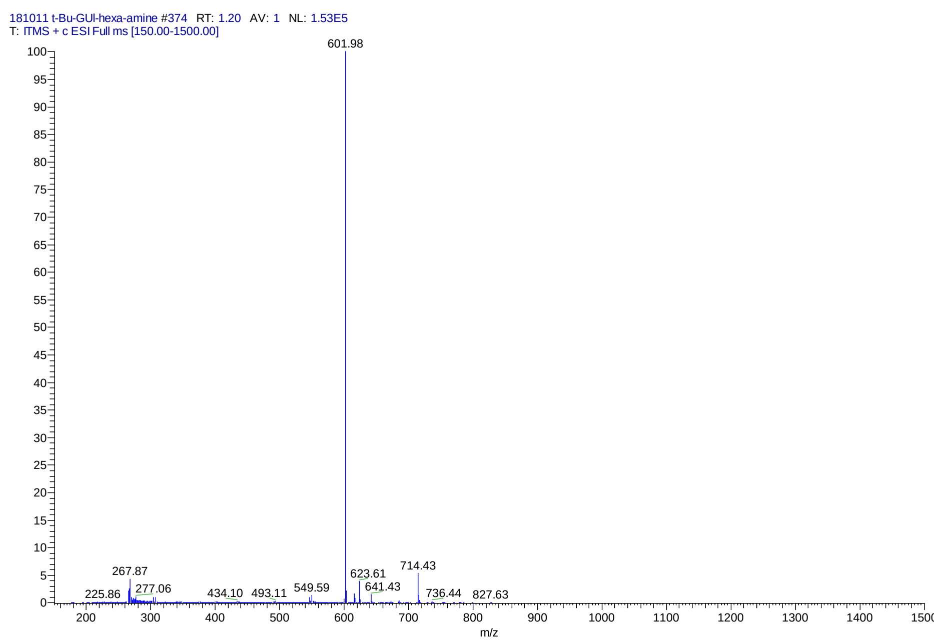

Figure S8: ESI-MS spectrum of **4**

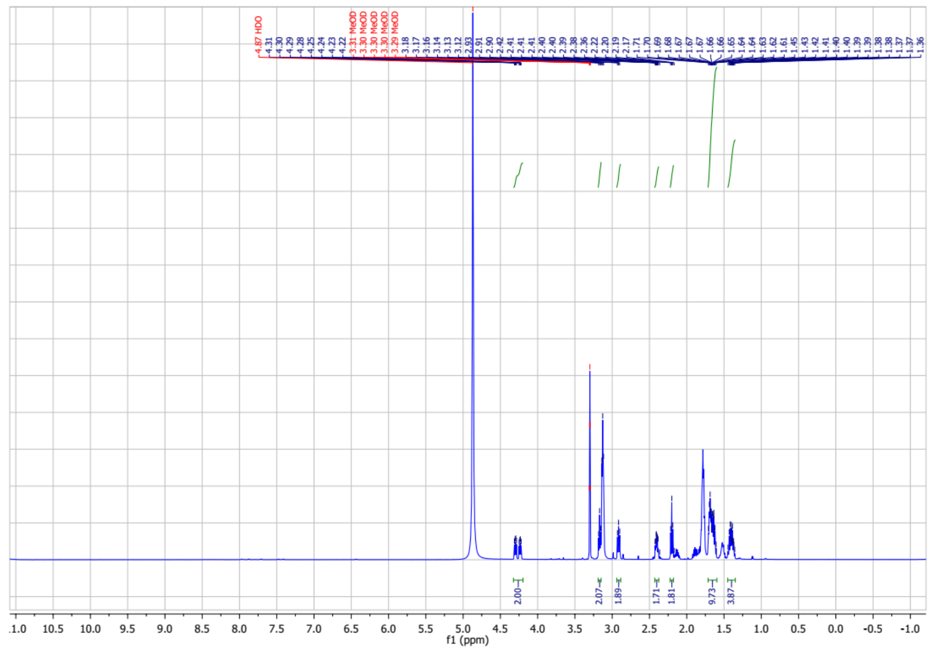

Figure S9. [1]H-NMR of **5**

Figure S10: ESI-MS spectrum of **5**

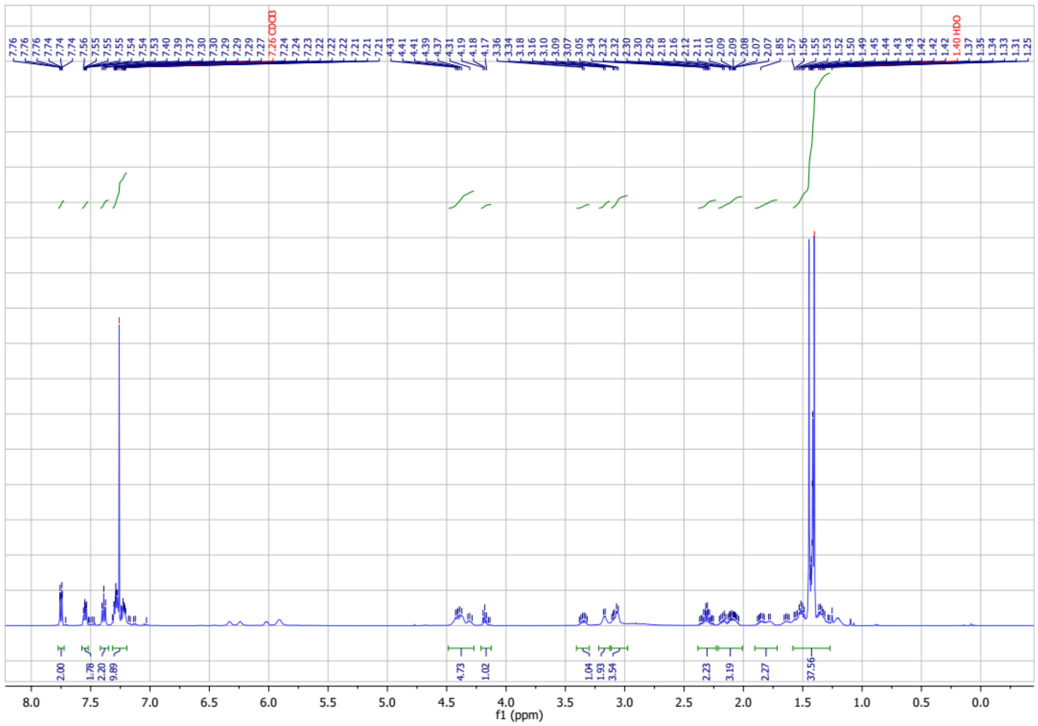

Figure S11. [1]H-NMR of **6**

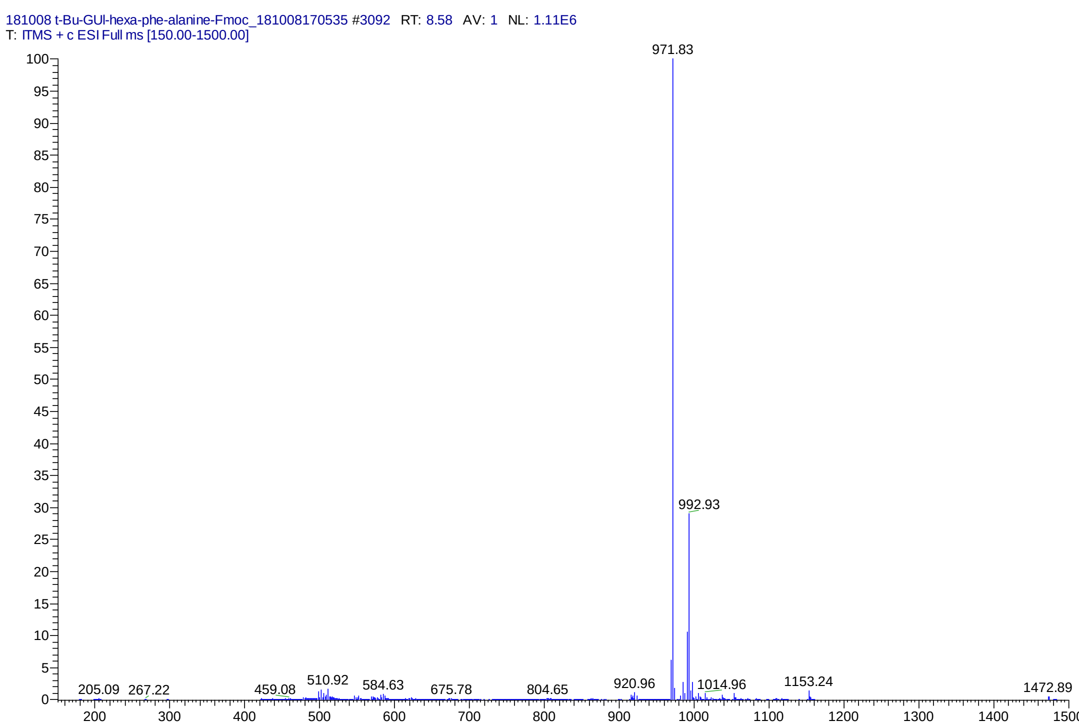

Figure S12: ESI-MS spectrum of **6**

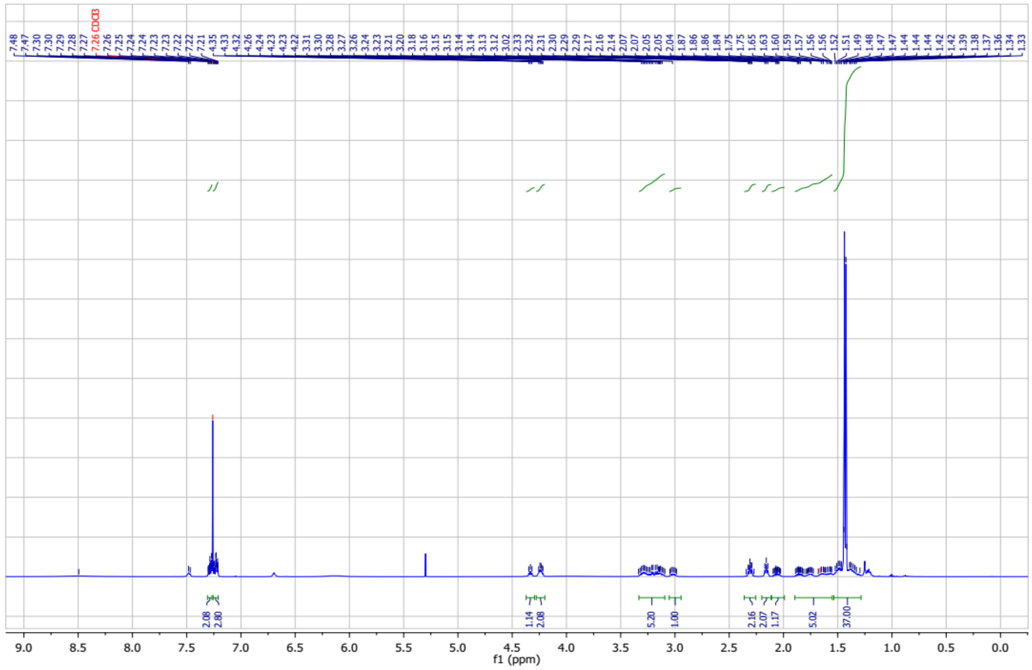

Figure S13. ¹H-NMR of **7**

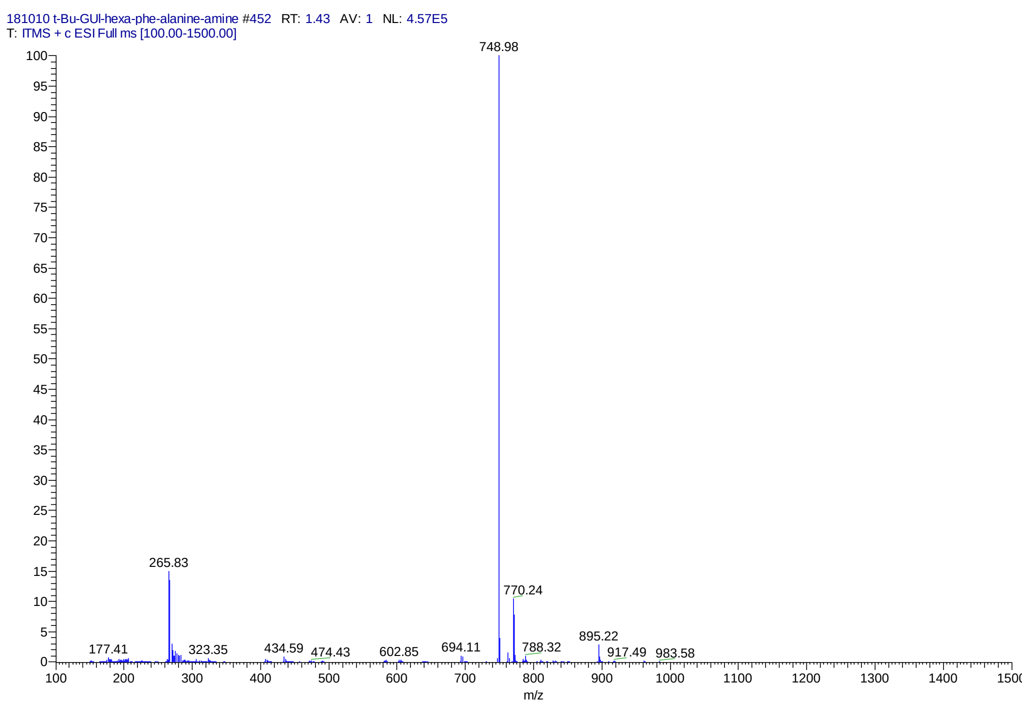

Figure S14: ESI-MS spectrum of **7**

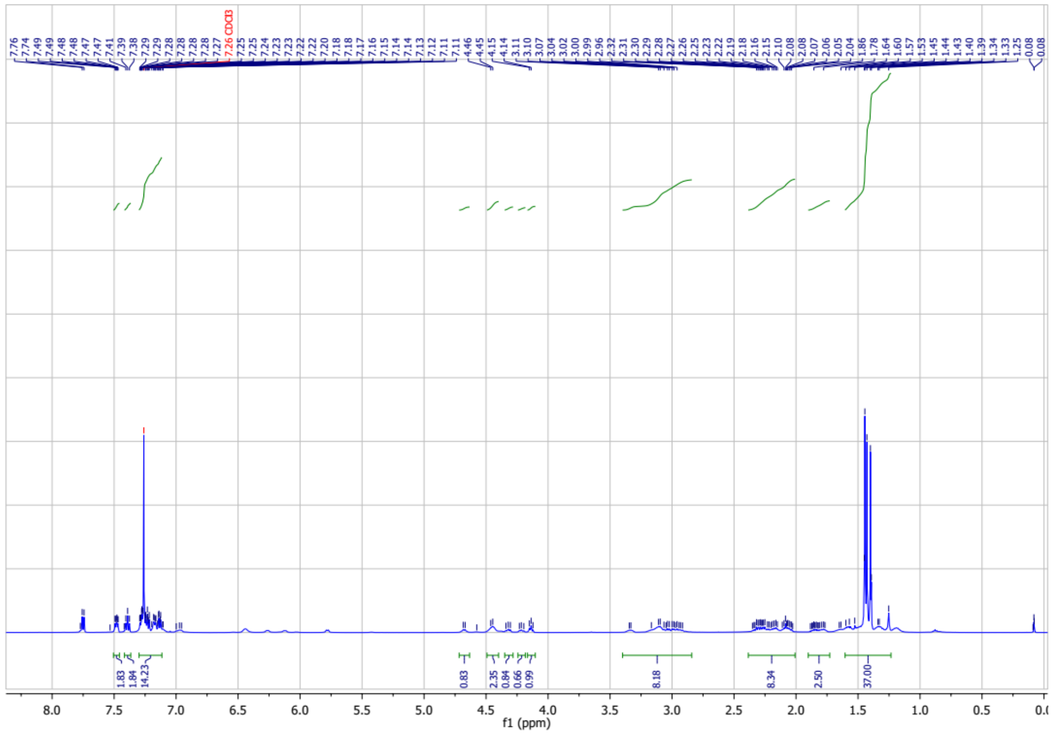

Figure S15. [1]H-NMR of **8**

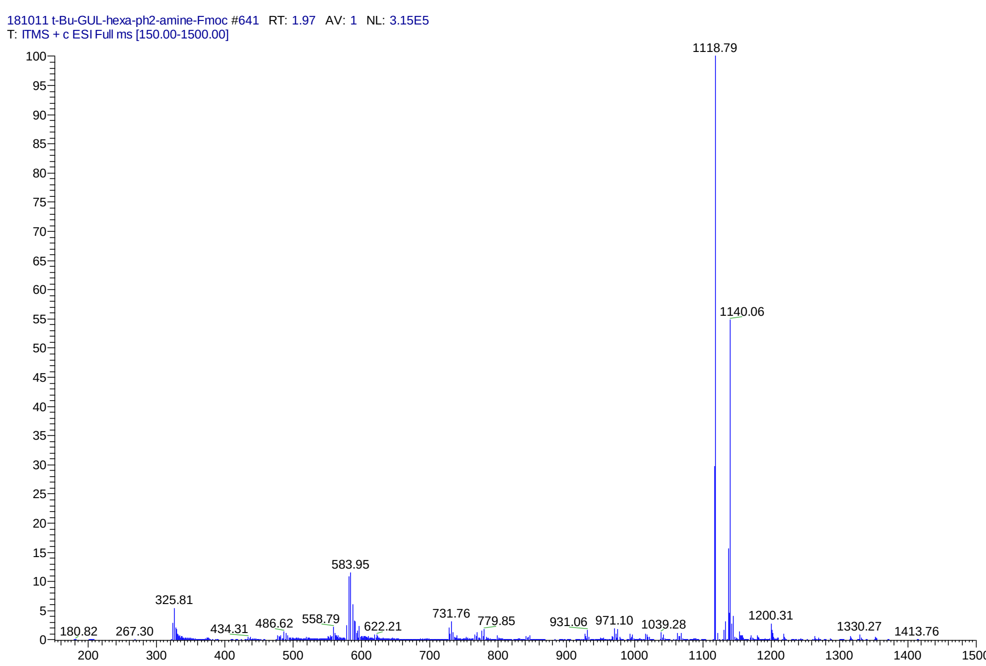

Figure S16: ESI-MS spectrum of **8**

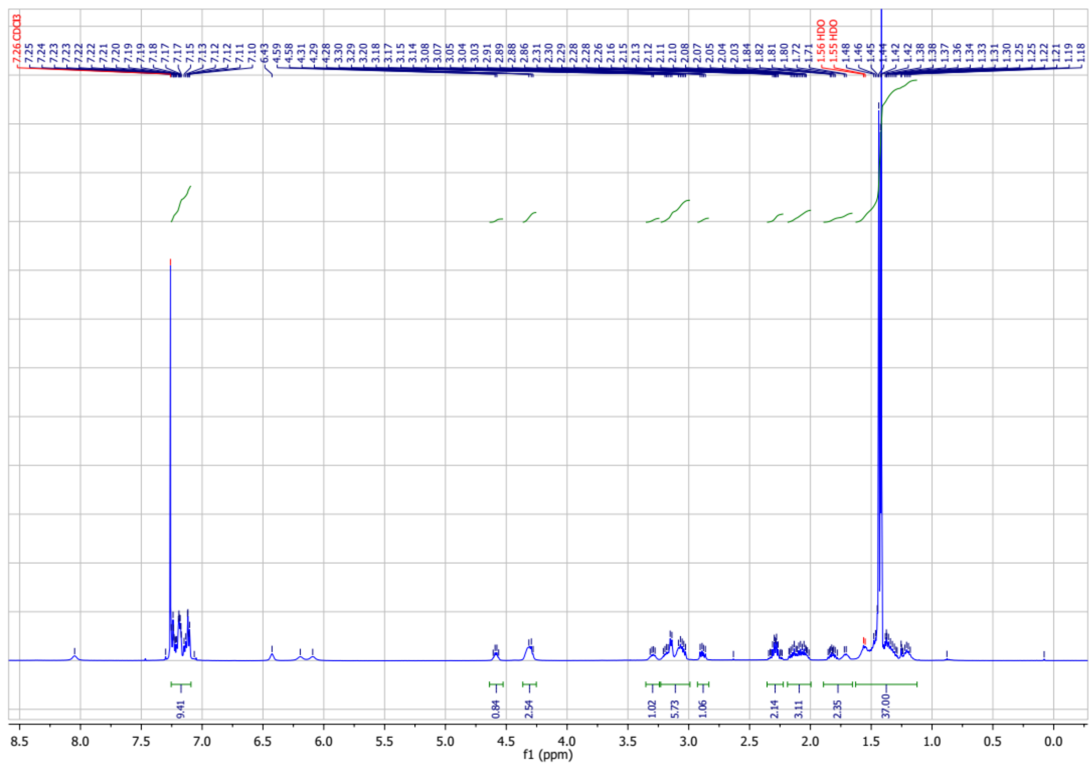

Figure S17. [1]H-NMR of **9**

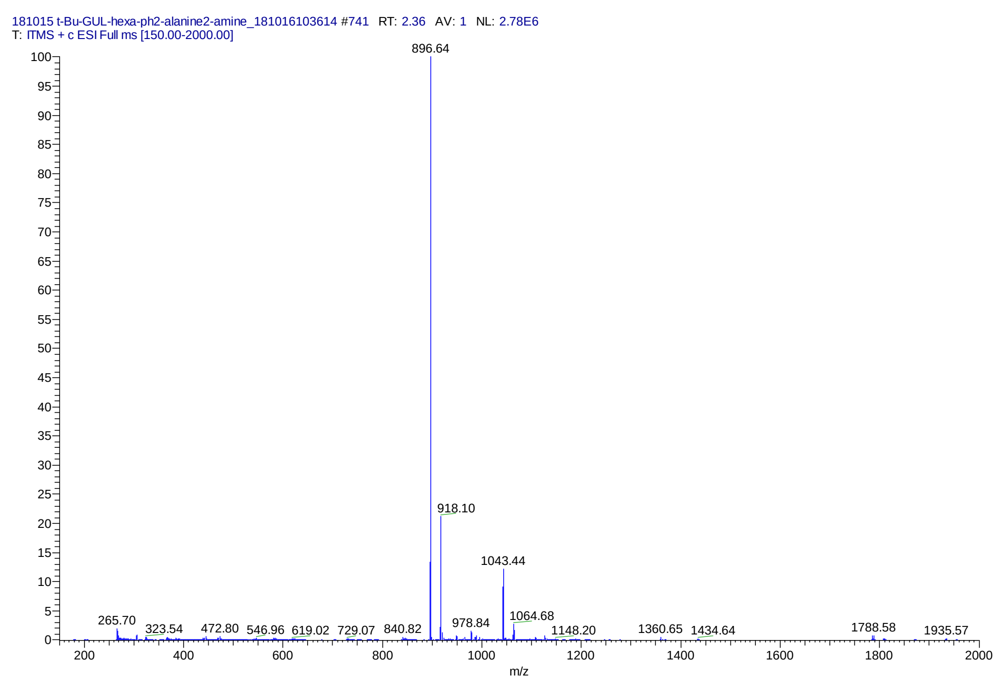

Figure S18: ESI-MS spectrum of **9**

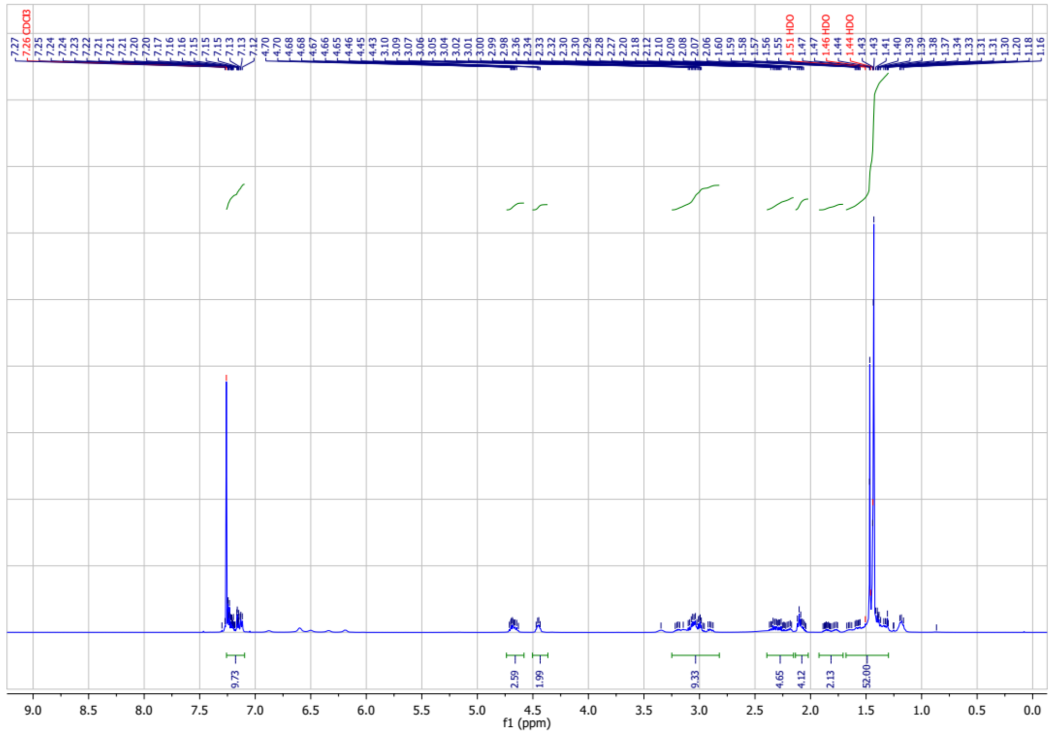

Figure S19. [1]H-NMR of **10**

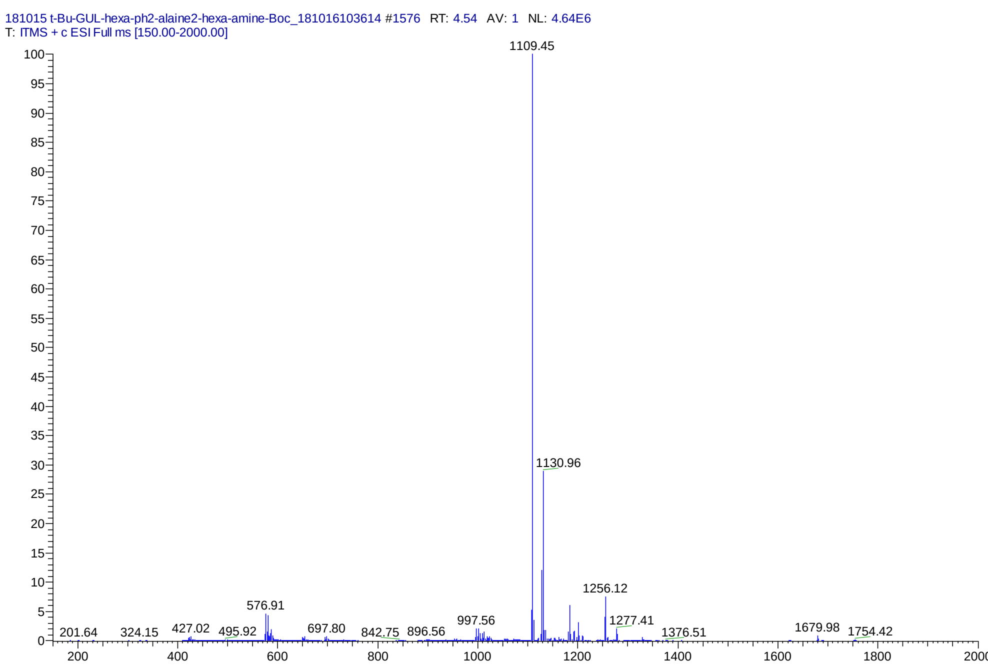

Figure S20: ESI-MS spectrum of **10**

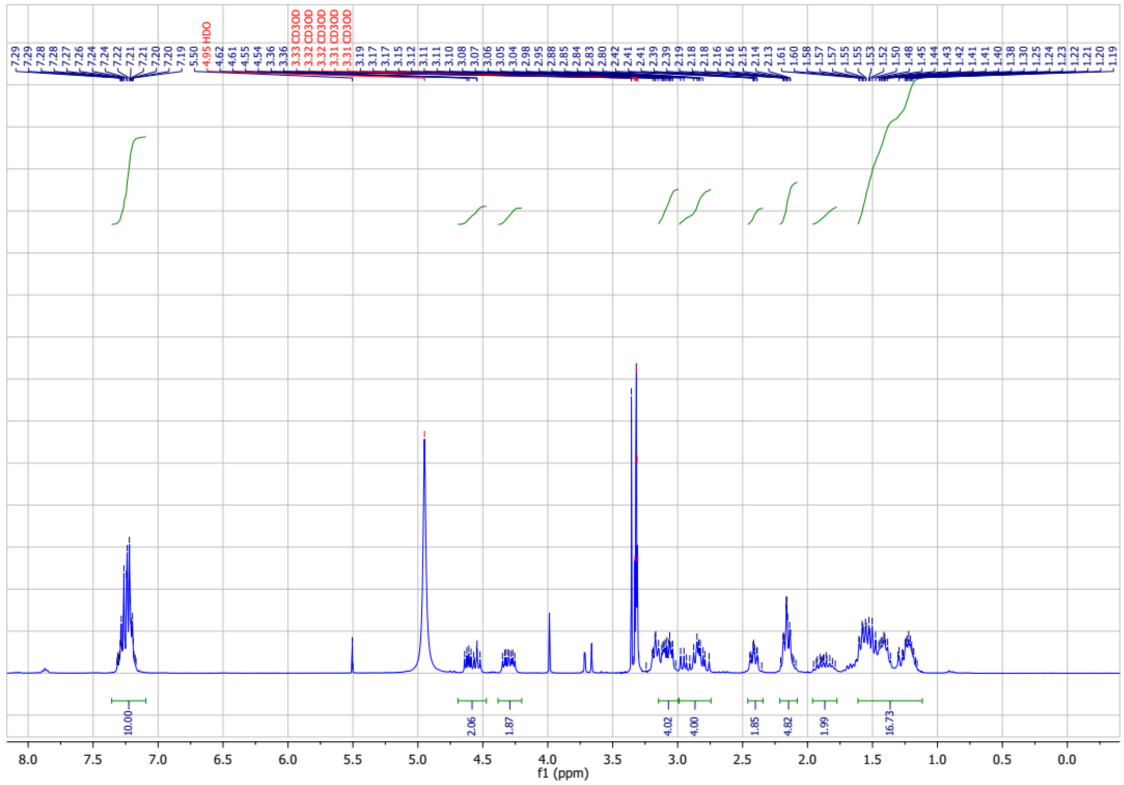

Figure S21. [1]H-NMR of **11**

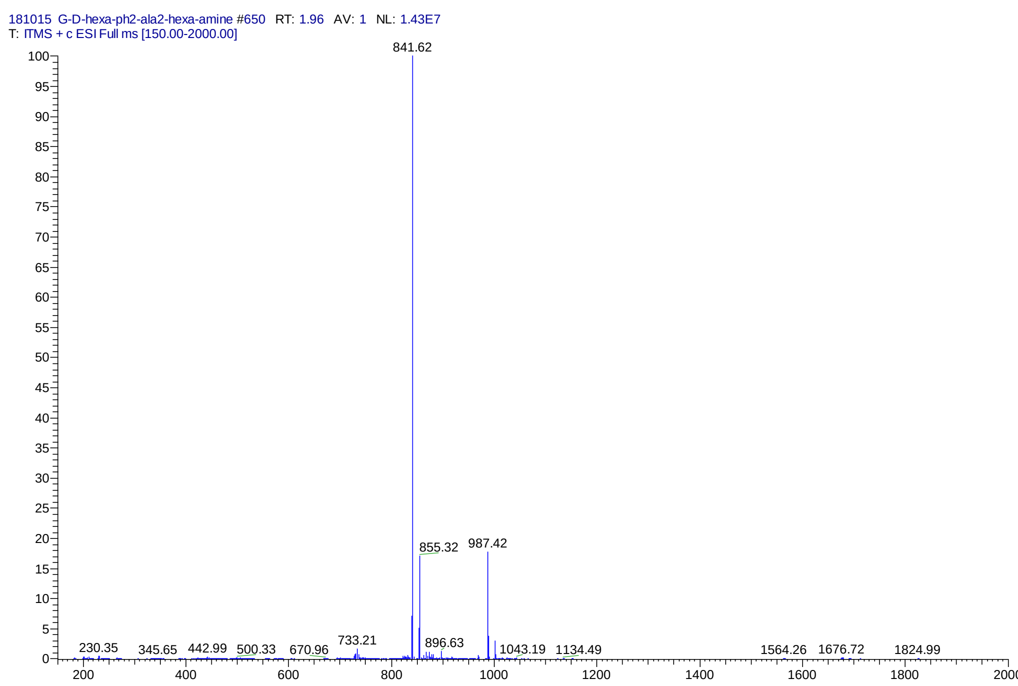

Figure S22: ESI-MS spectrum of **11**

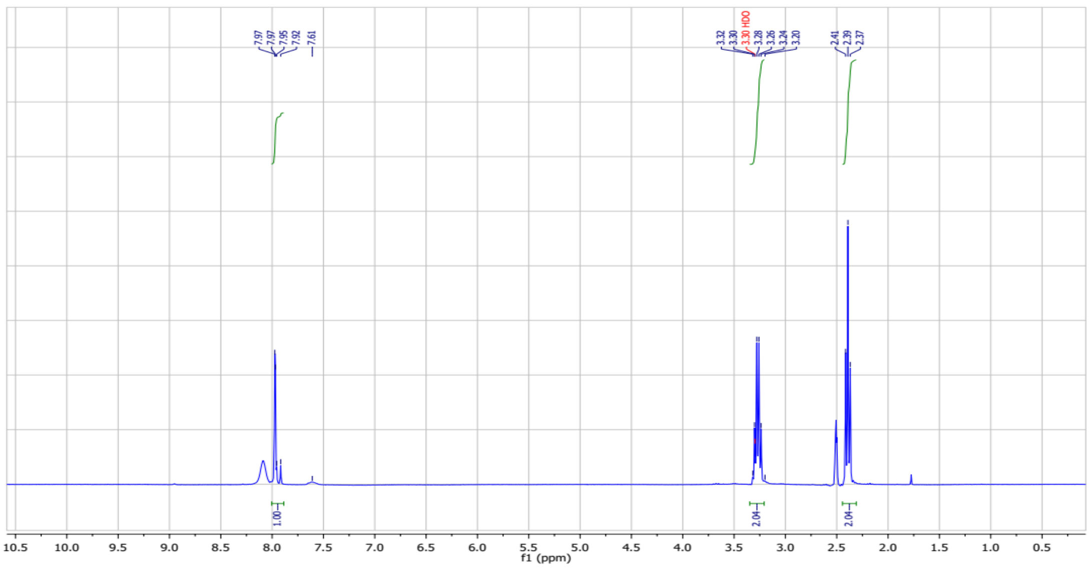

Figure S23. [1]H-NMR compound **12**

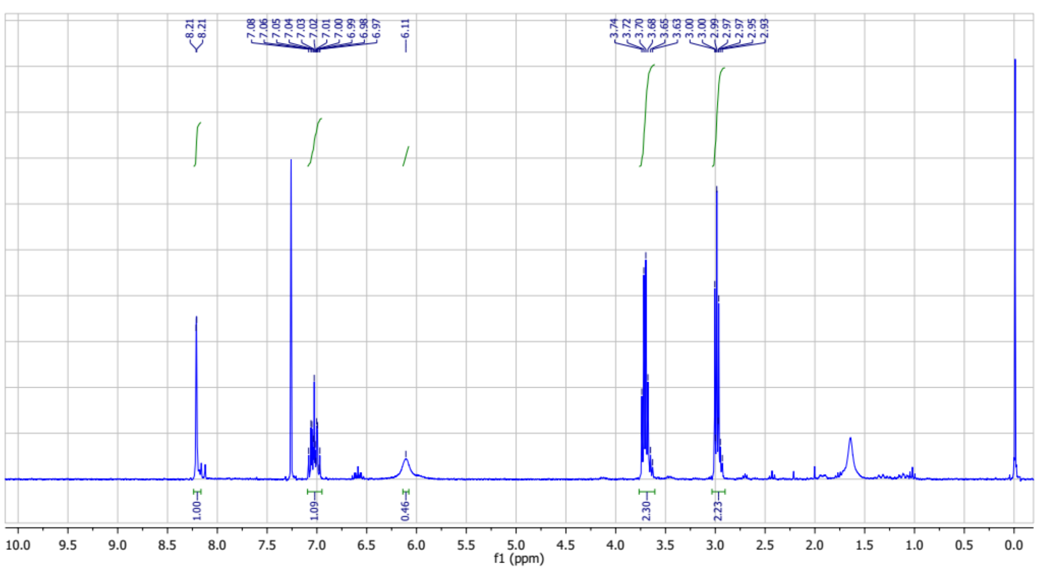

Figure S24. [1]H-NMR of compound **13**

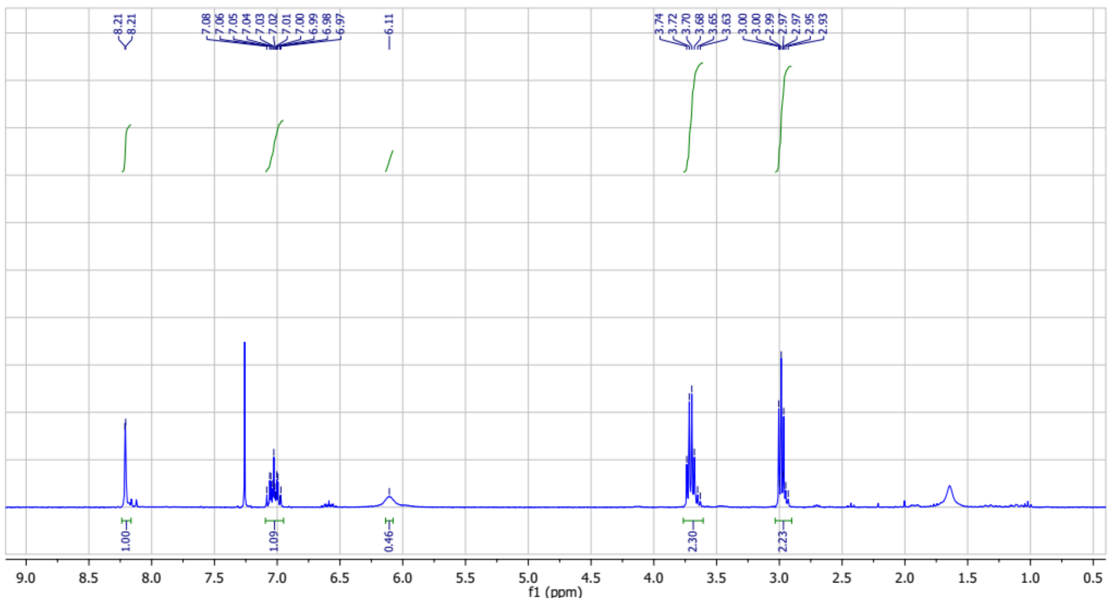

Figure S25. [1]H-NMR of compound **14**

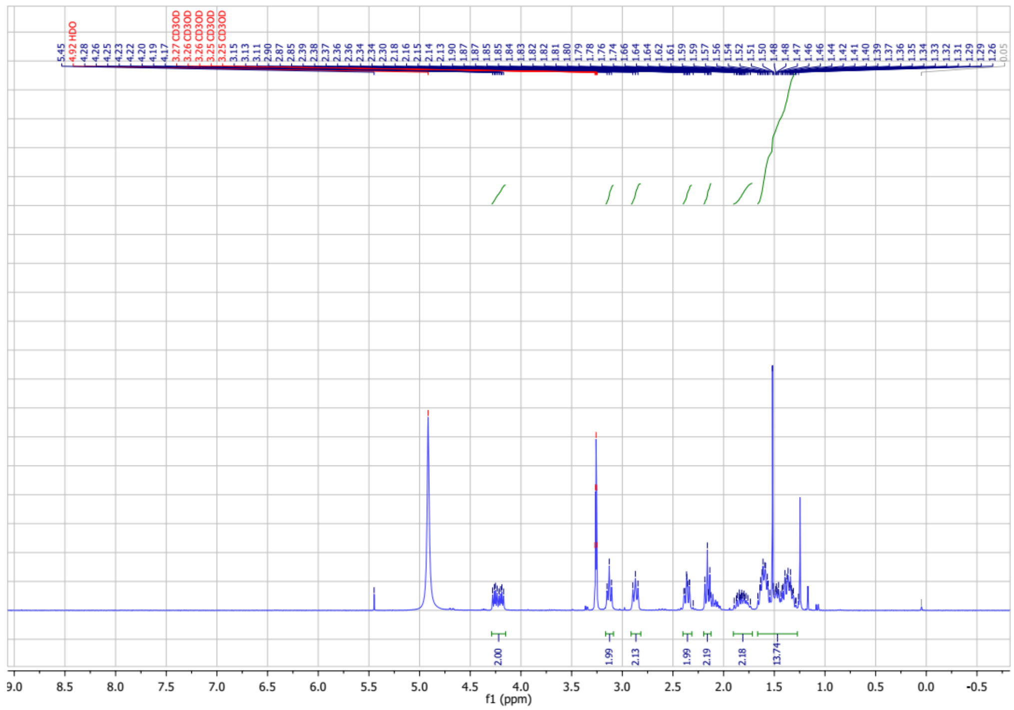

Figure S26. [1]H-NMR of compound **15**

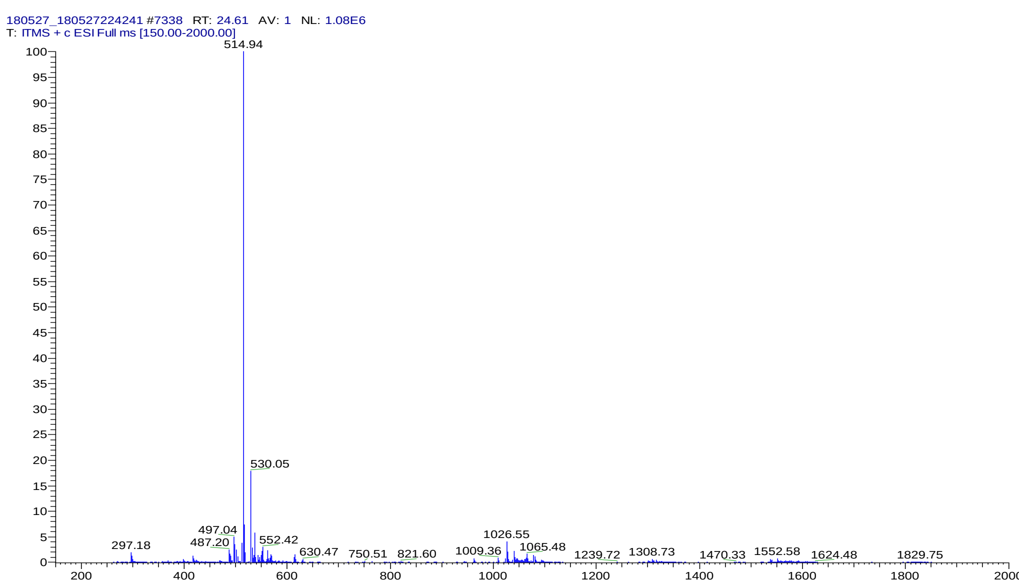

Figure S27: ESI-MS spectrum of **15**

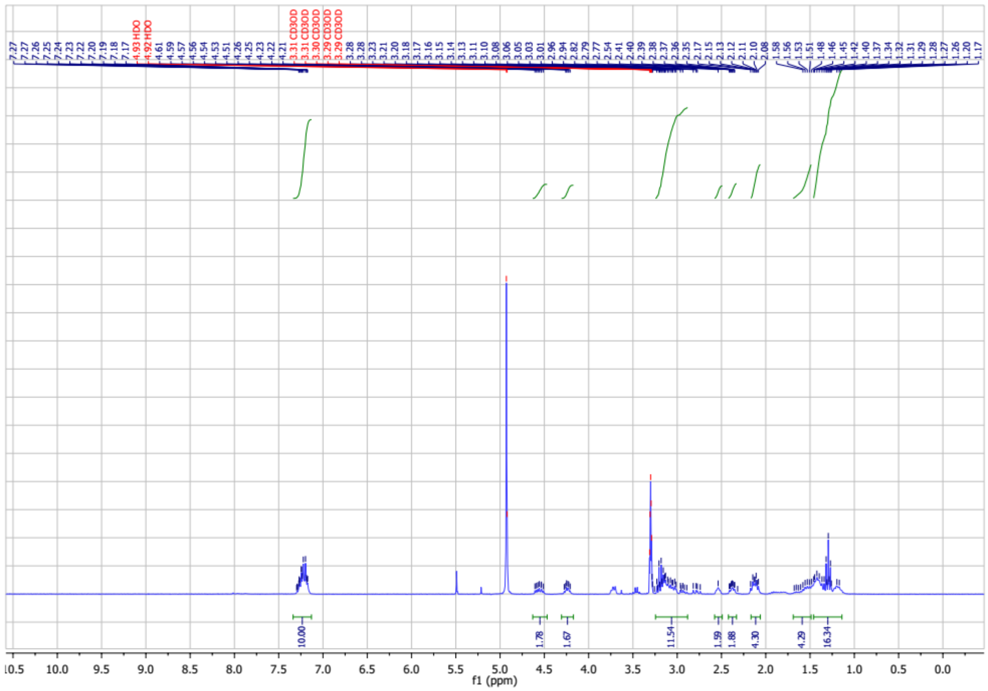

Figure S28. [1]H-NMR of **16**

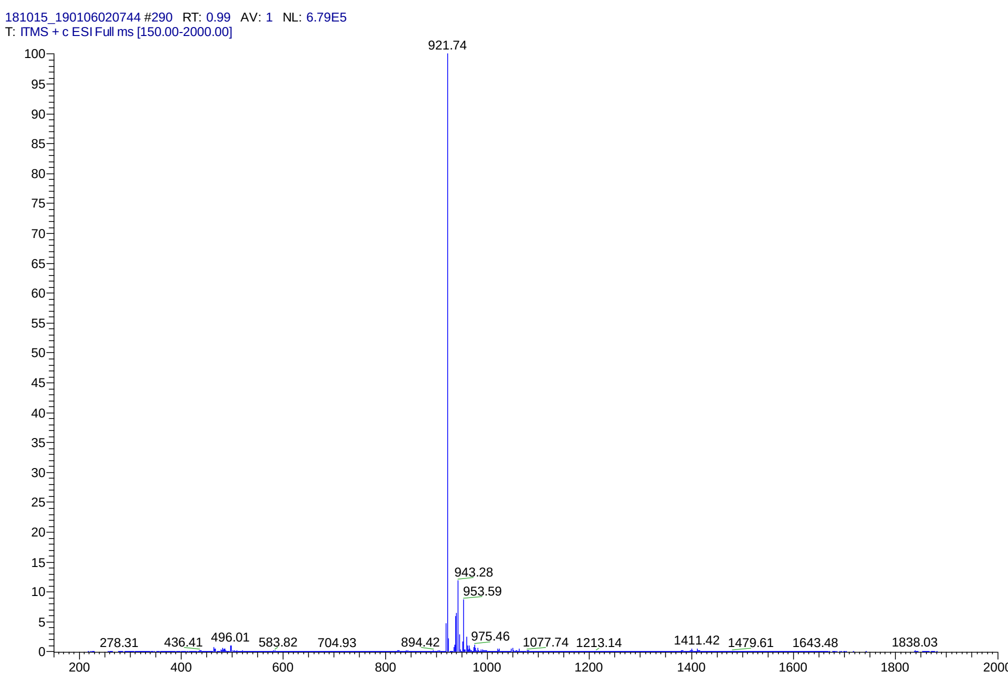

Figure S29: ESI-MS spectrum of **16**

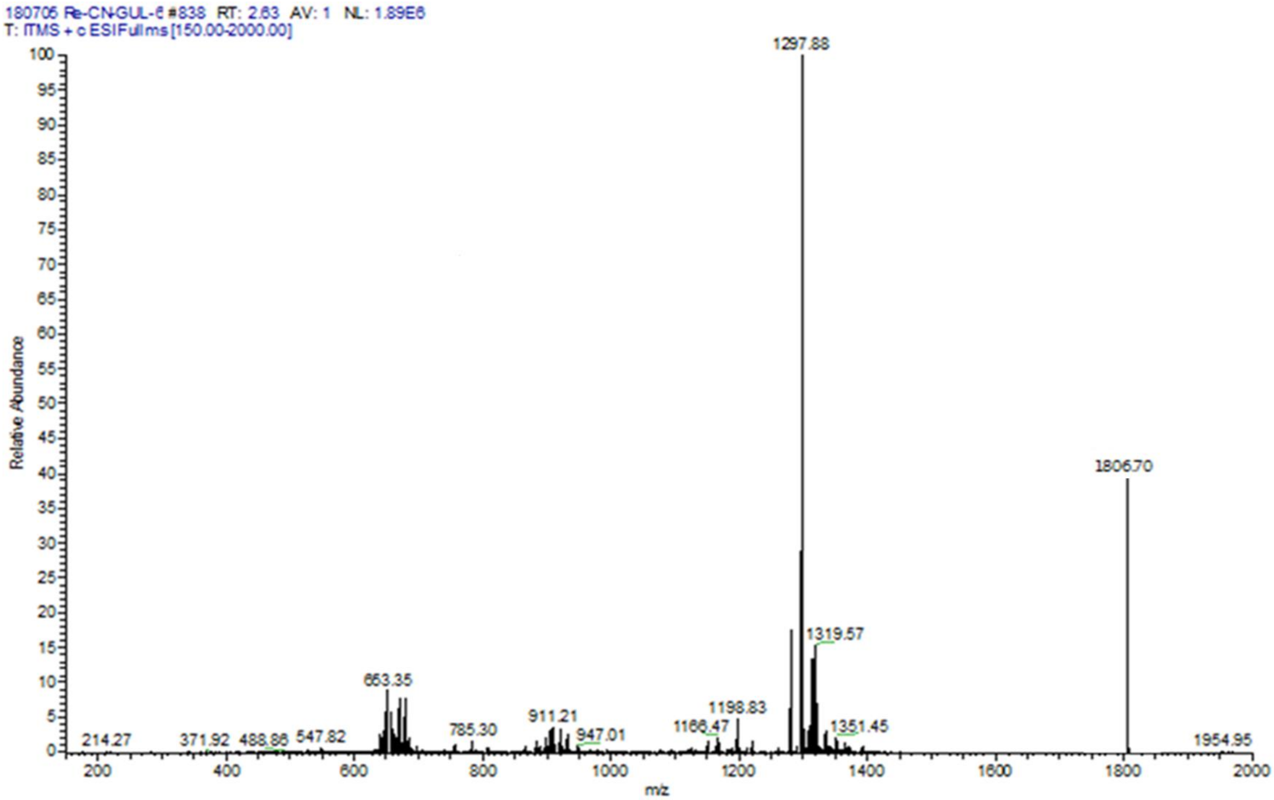

Figure S30: ESI-MS spectrum of Re-**15**

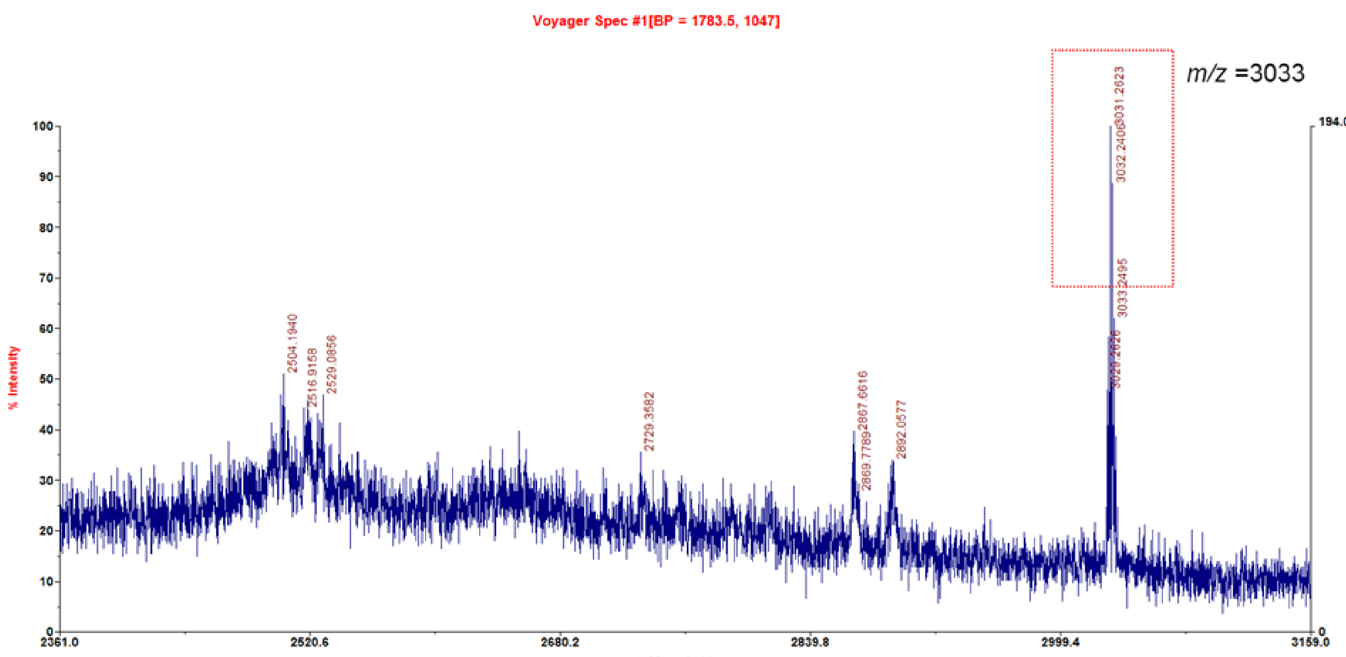

Figure S31: MALDI-TOF spectrum of Re-**16**