Supporting Information

Development of novel Pt(IV)-Carbohydrate derivatives as targeted anticancer agents against Osteosarcoma

Eoin Moynihan, Silvia Panseri, Giada Bassi, Arianna Rossi , Elisabetta Campodoni, Eithne Dempsey, Monica Montesi, Trinidad Velasco Torrijos, Diego Montagner

NMR Spectra

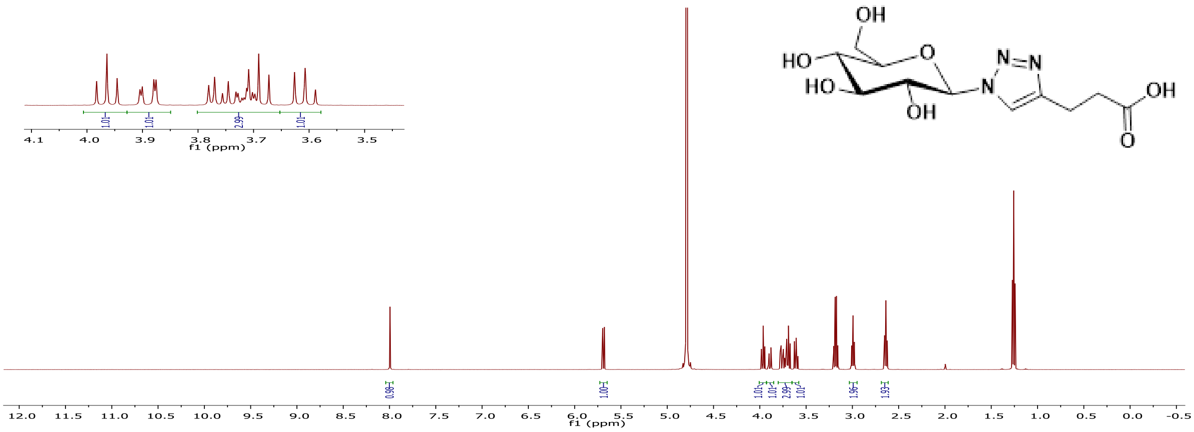

Figure S1a: ^1H NMR spectrum of **11** in D_2O

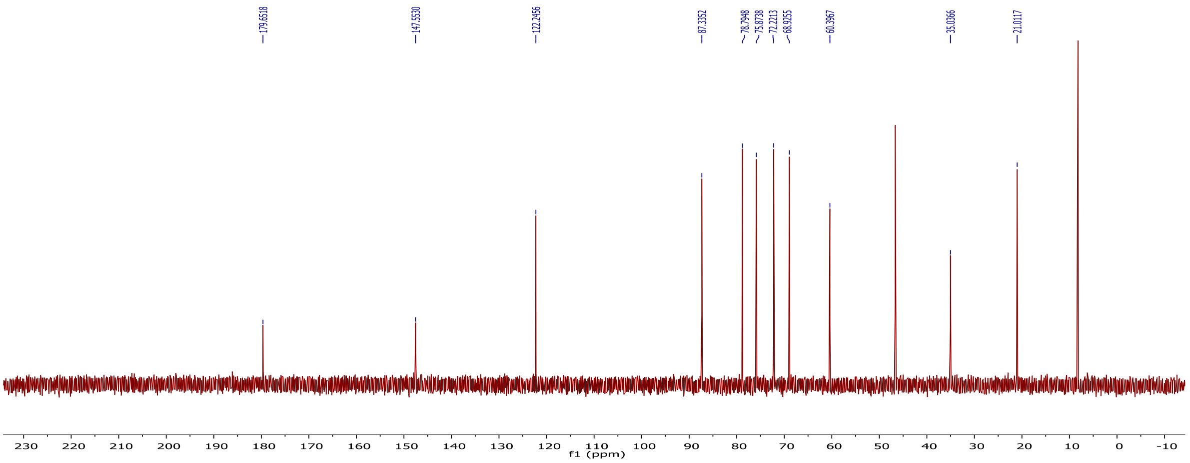

Figure S1b: ^{13}C NMR spectrum of **11** in D_2O

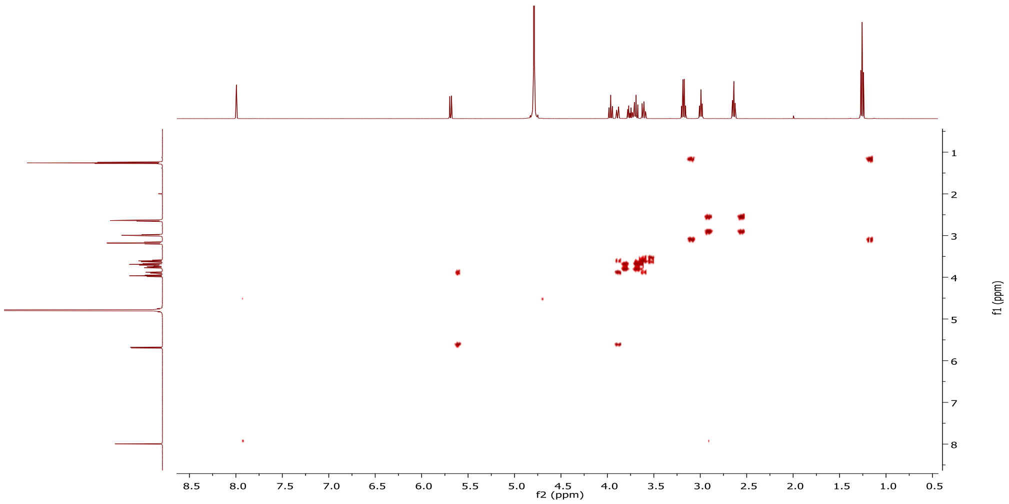

Figure S1c: COSY NMR spectrum of **11** in D_2O

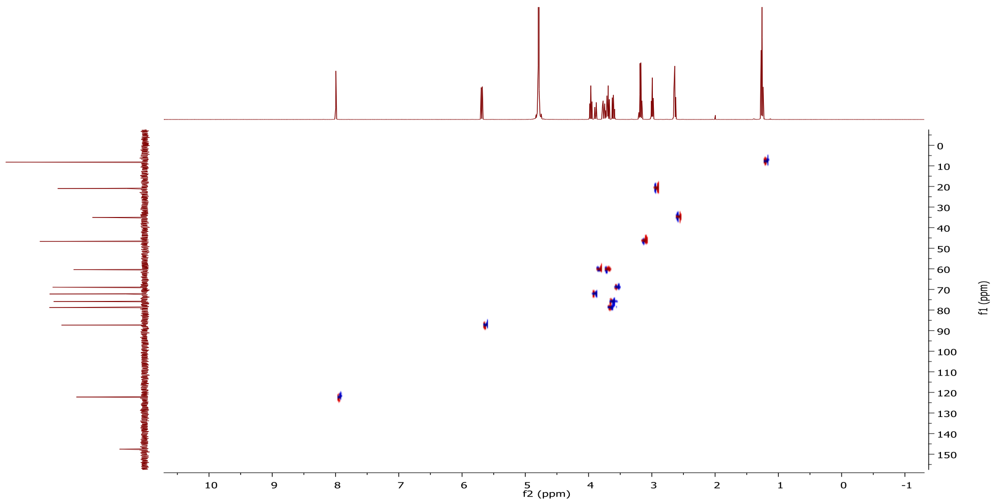

Figure S1d: HSQC NMR spectrum of **11** in D_2O

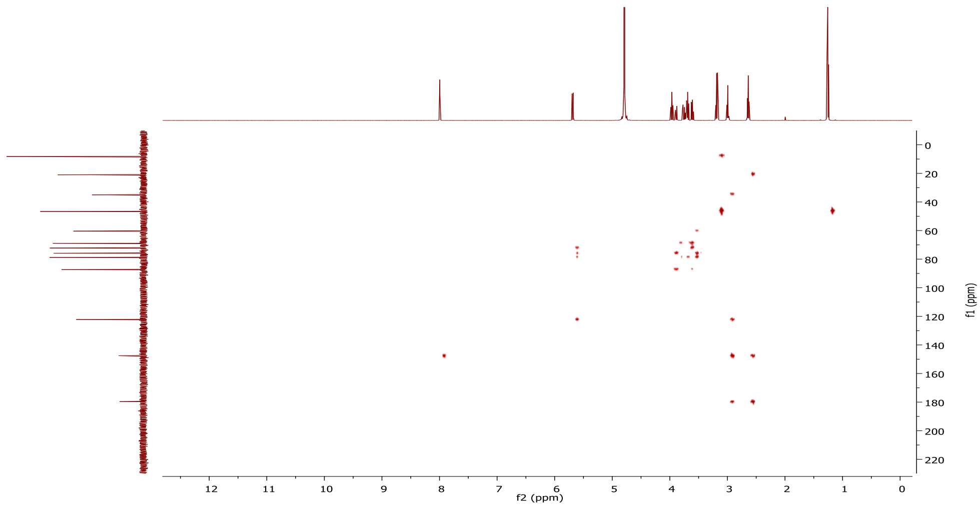

Figure S1e: HMBC NMR spectrum of **11** in D_2O

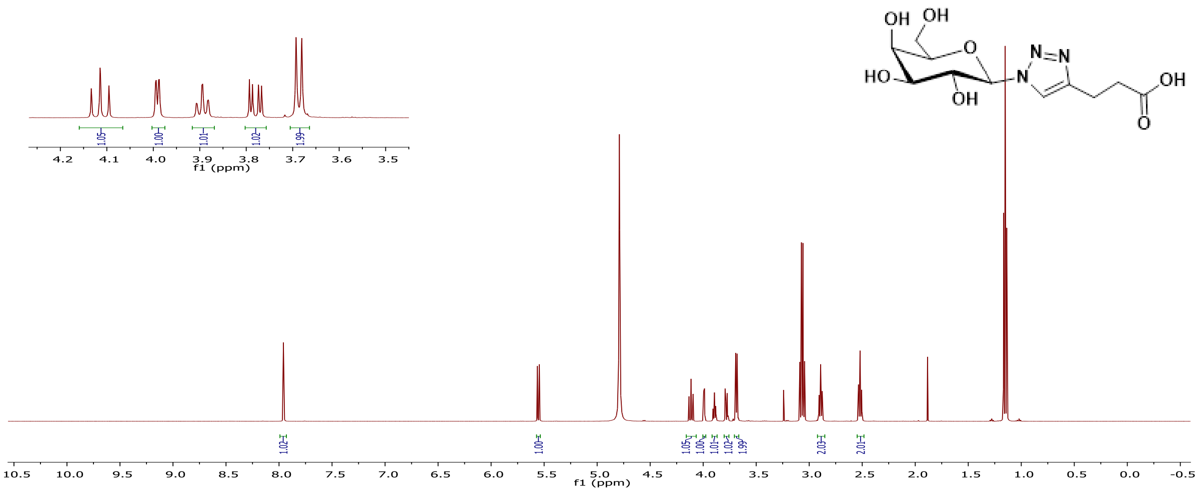

Figure S2a: ^{1}H NMR spectrum of **12** in D_2O

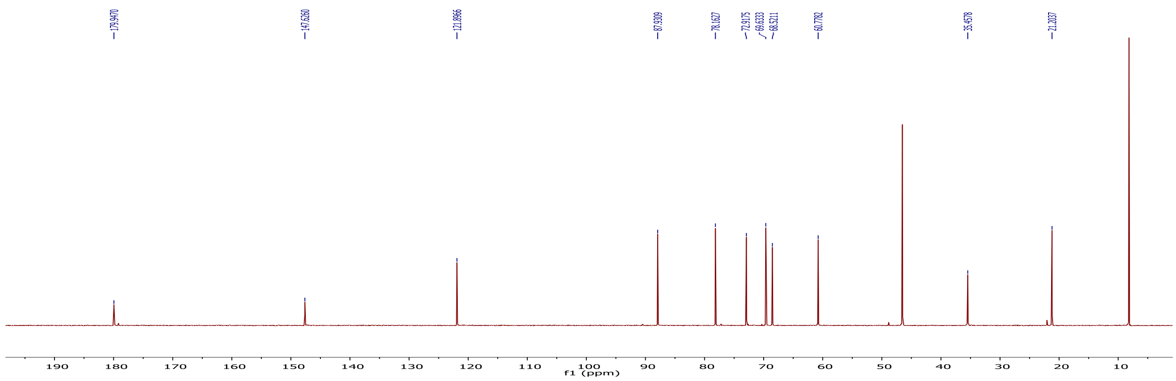

Figure S2b: ^{13}C NMR spectrum of **12** in D_2O

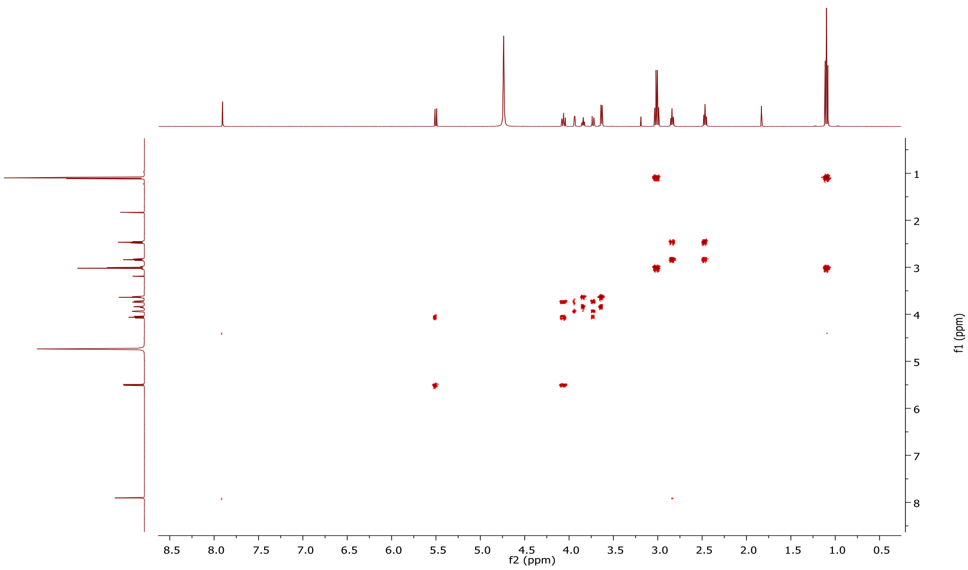

Figure S2c: COSY NMR spectrum of **12** in D_2O

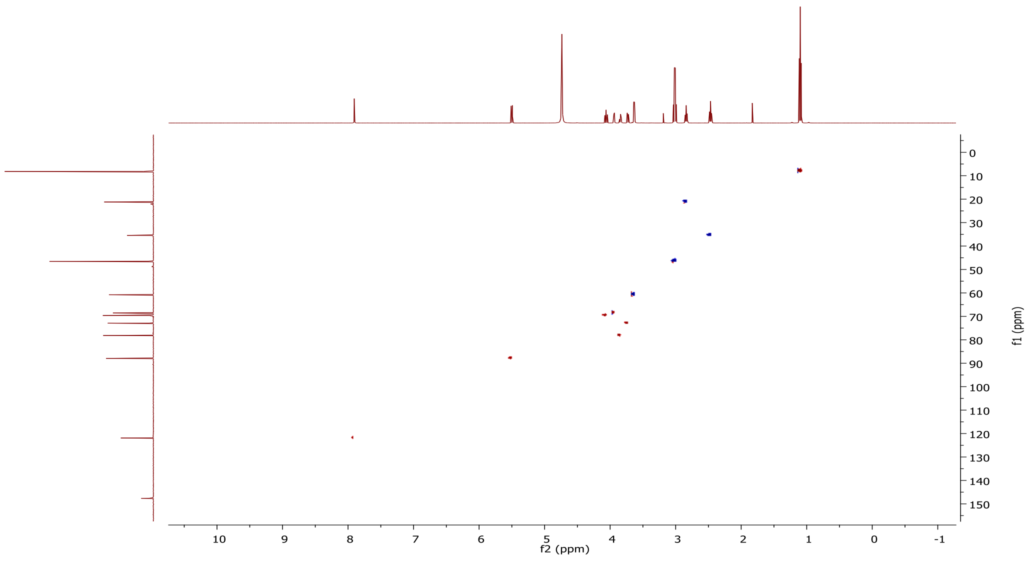

Figure S2d: HSQC NMR spectrum of **12** in D_2O

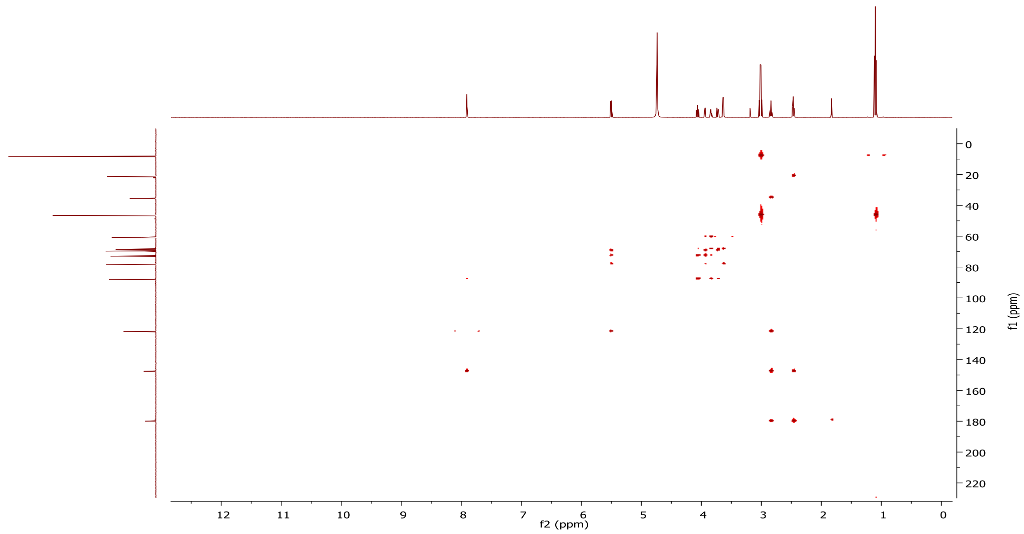

Figure S2e: HMBC NMR spectrum of **12** in D_2O

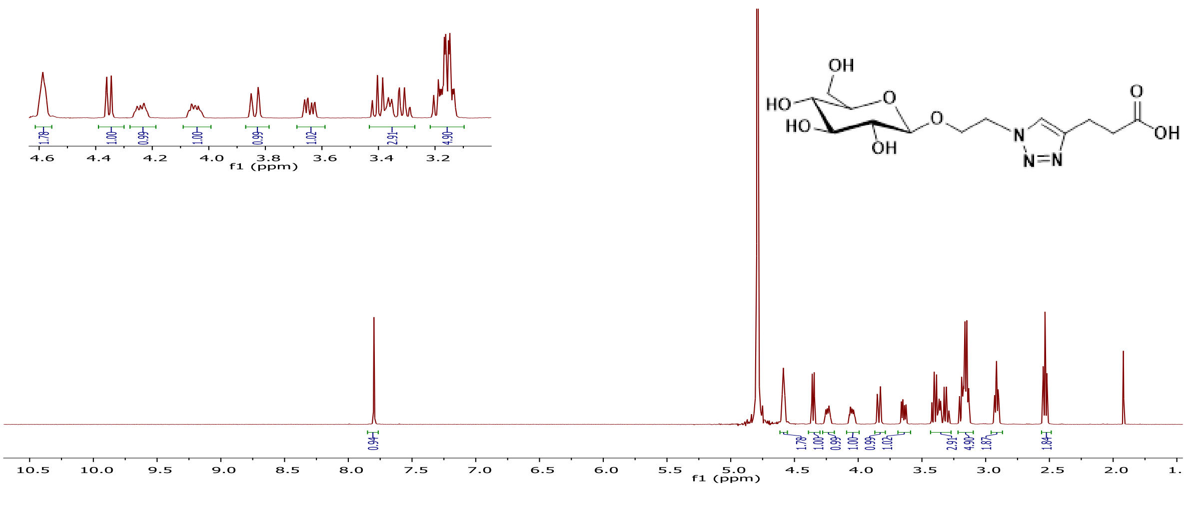

Figure S3a: ^{1}H NMR spectrum of **21** in D_2O

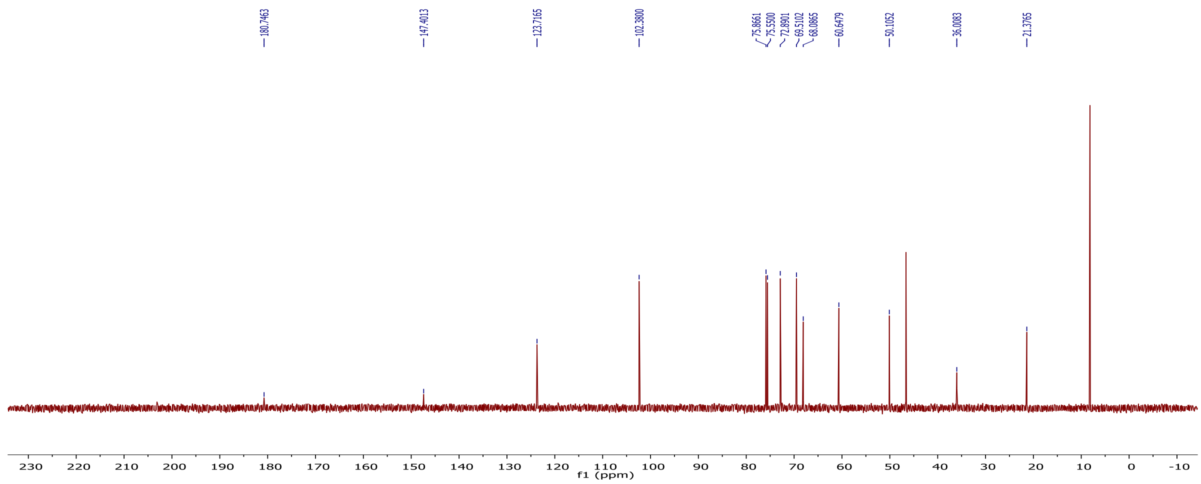

Figure S3b: ^{13}C NMR spectrum of **21** in D_2O

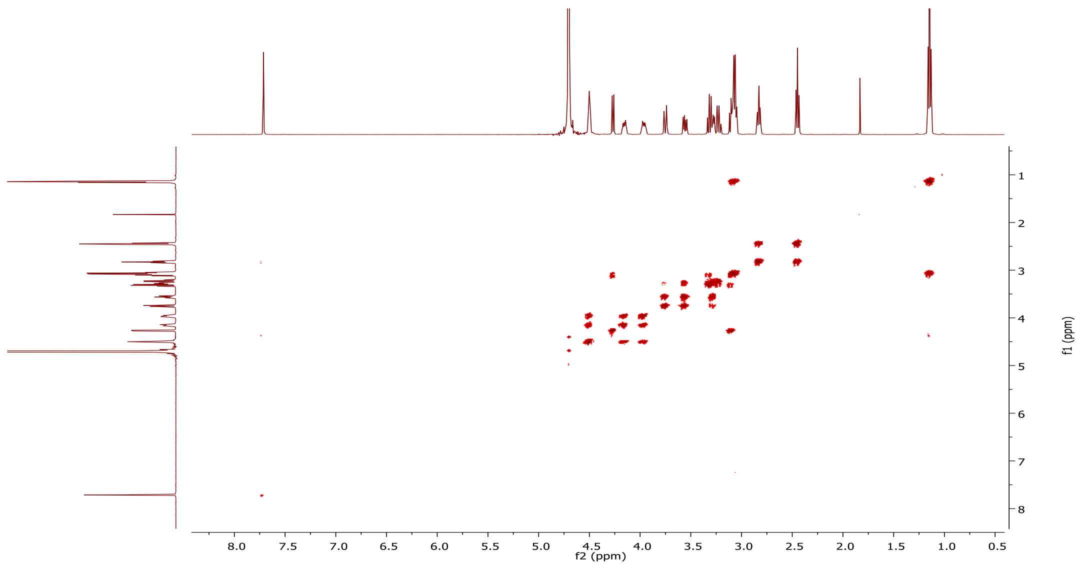

Figure S3c: COSY NMR spectrum of **21** in D_2O

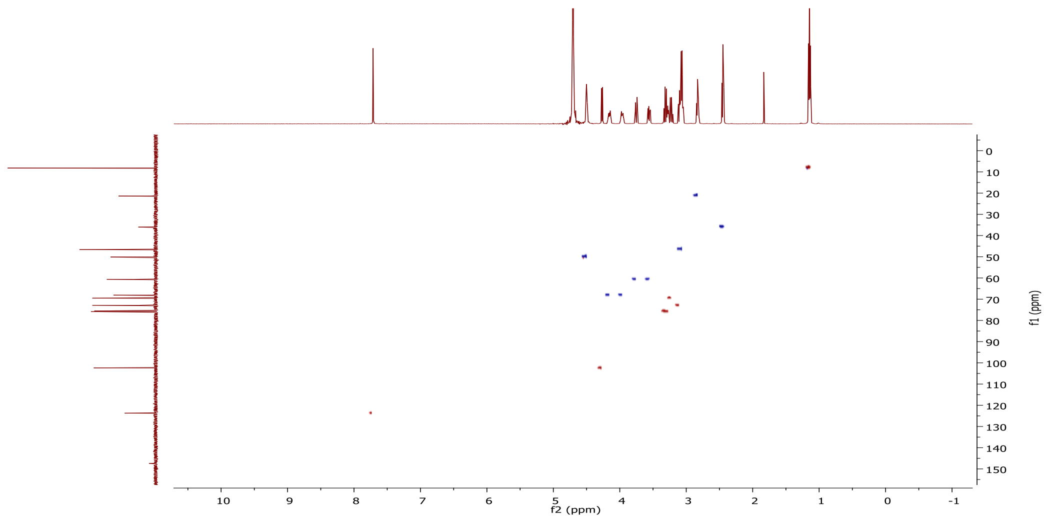

Figure S3d: HSQC NMR spectrum of **21** in D_2O

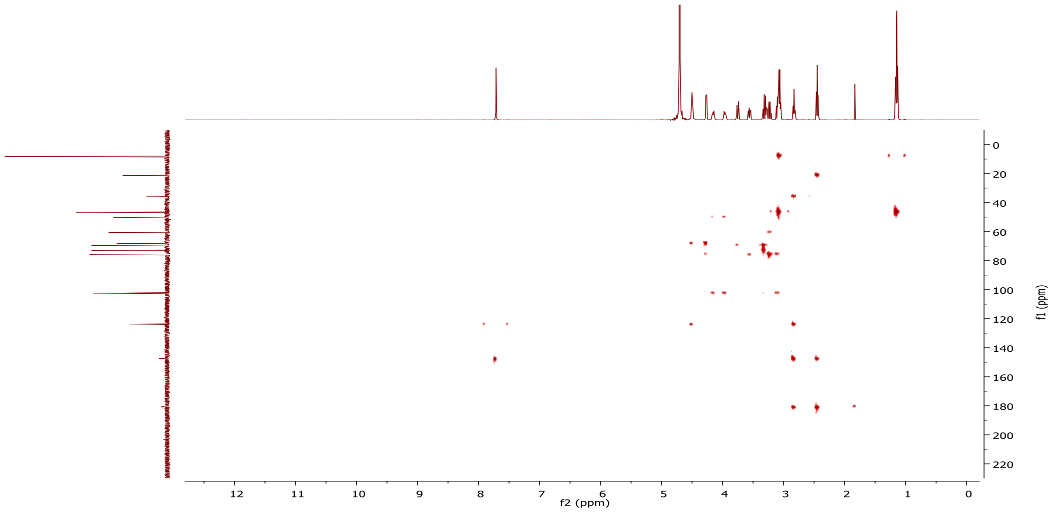

Figure S3e: HMBC NMR spectrum of **21** in D_2O

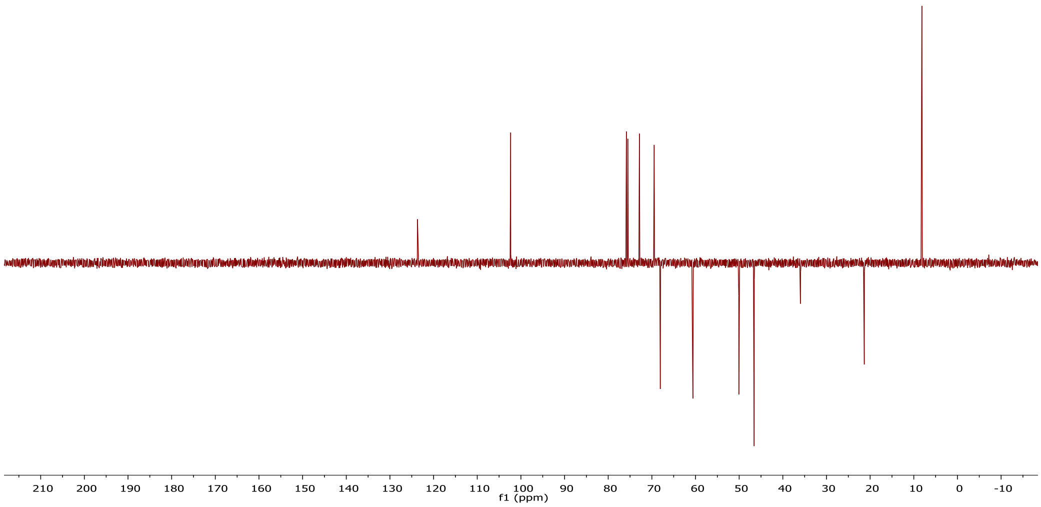

Figure S3f: DEPT-135 NMR spectrum of **21** in D_2O

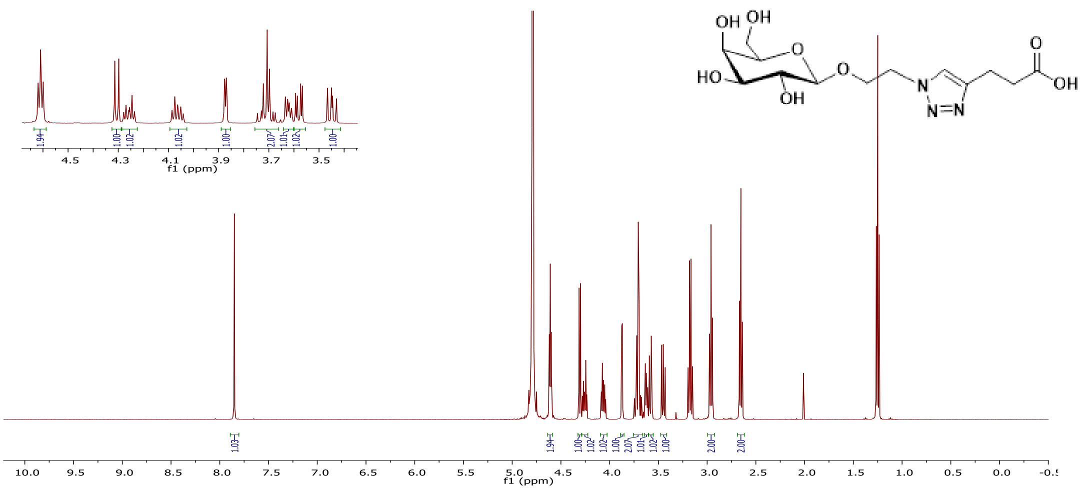

Figure S4a: ^1H NMR spectrum of **22** in D_2O

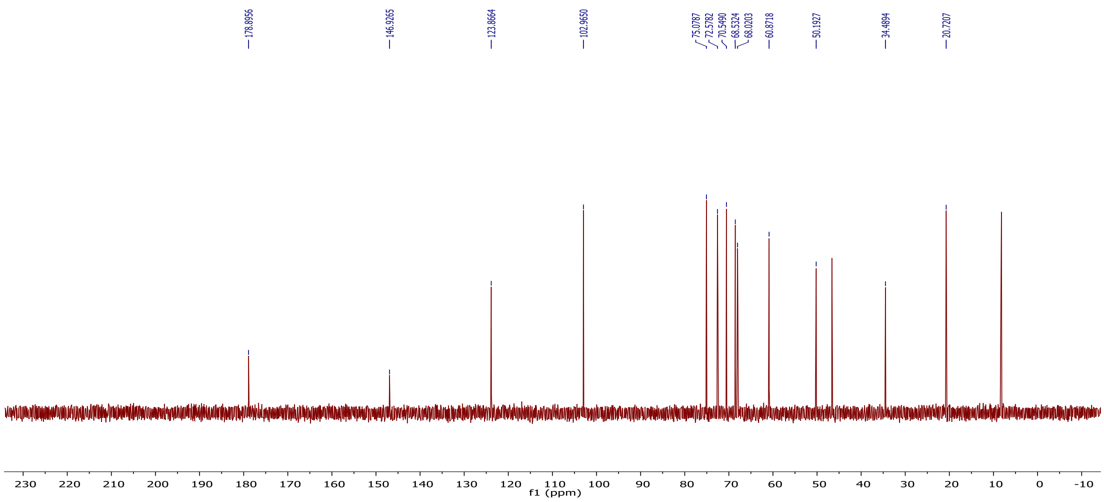

Figure S4b: ^{13}C NMR spectrum of **22** in D_2O

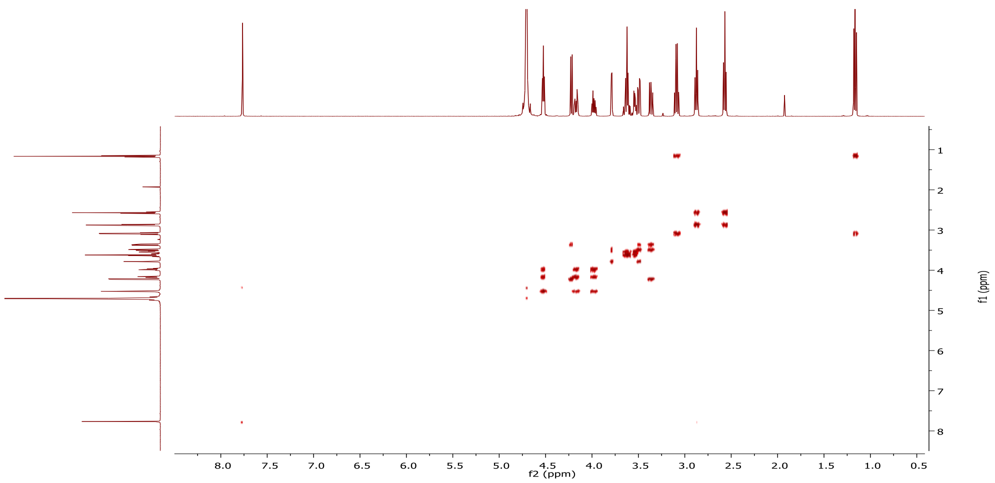

Figure S4c: COSY NMR spectrum of **22** in D_2O

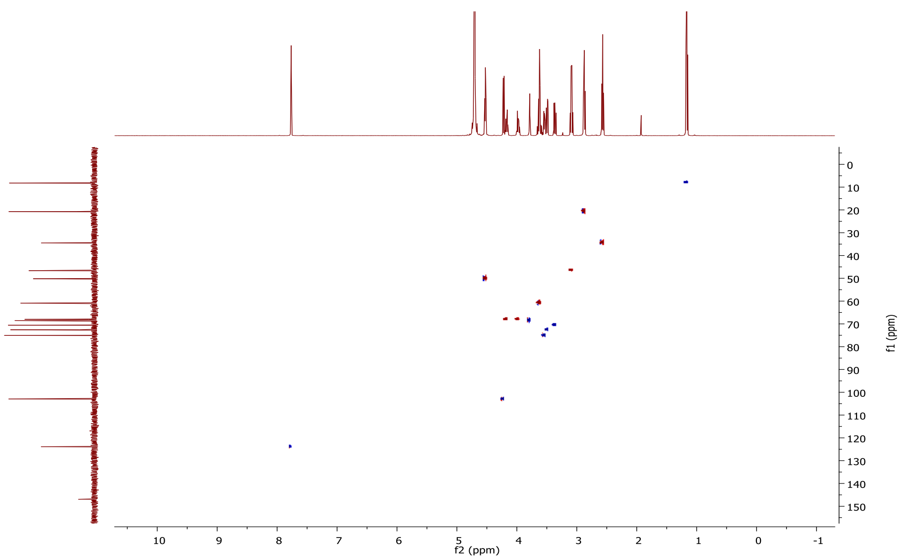

Figure S4d: HSQC NMR spectrum of **22** in D_2O

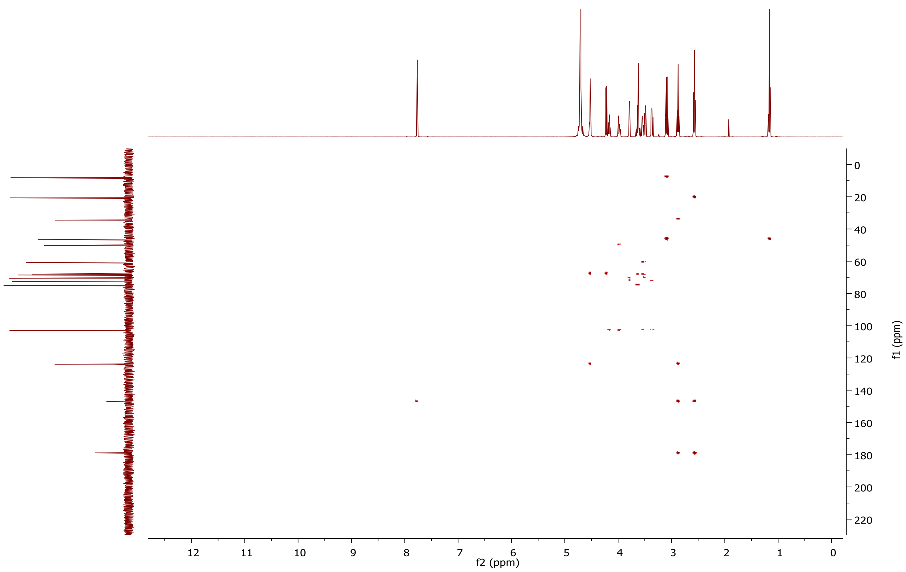

Figure S4e: HMBC NMR spectrum of **22** in D_2O

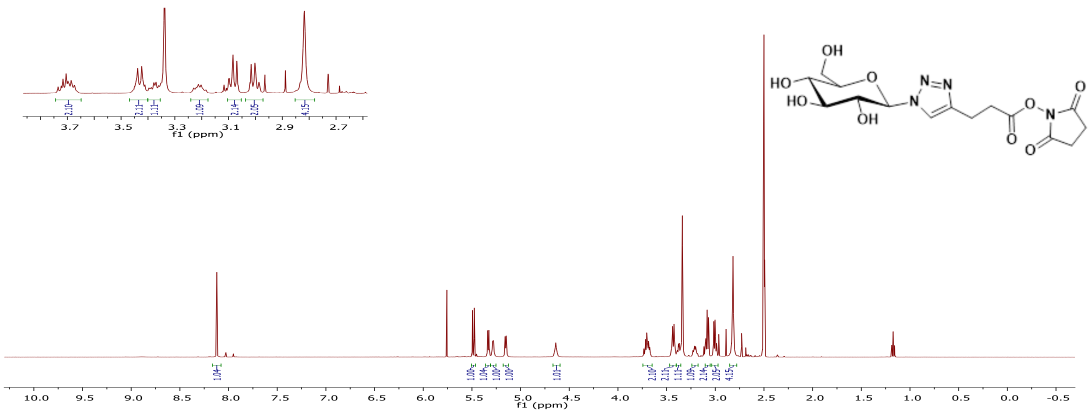

Figure S5a: ^{1}H NMR spectrum of **13** in DMSO

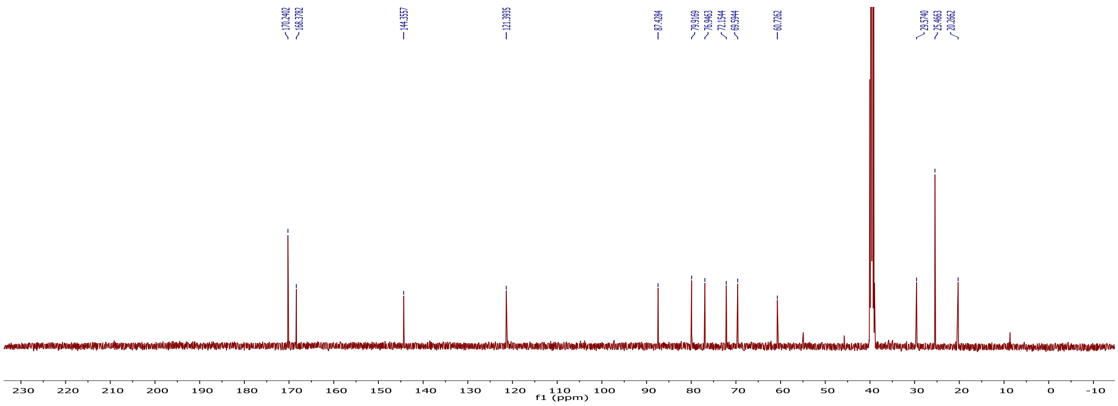

Figure S5b: ^{13}C NMR spectrum of **13** in DMSO

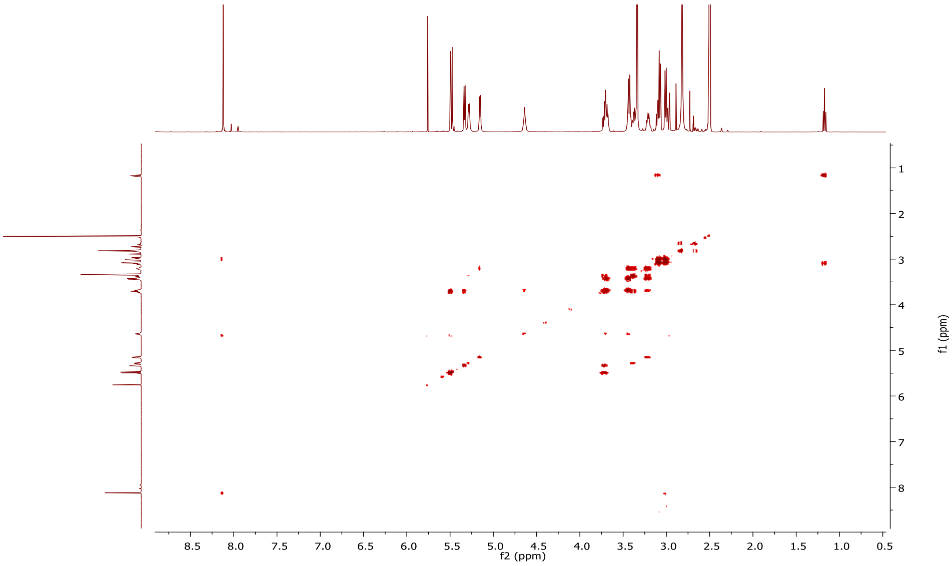

Figure S5c: COSY NMR spectrum of **13** in DMSO

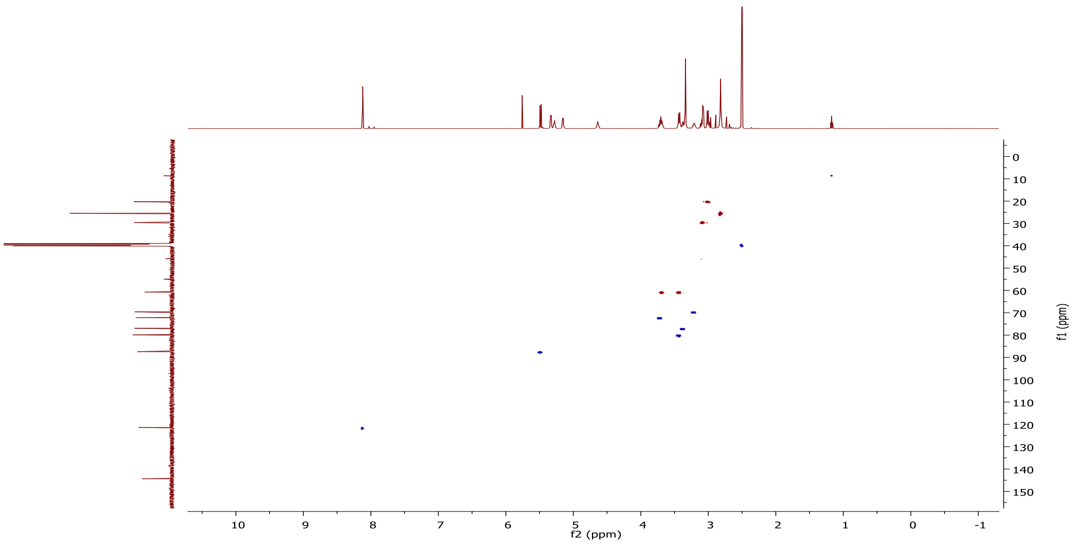

Figure S5d: HSQC NMR spectrum of **13** in DMSO

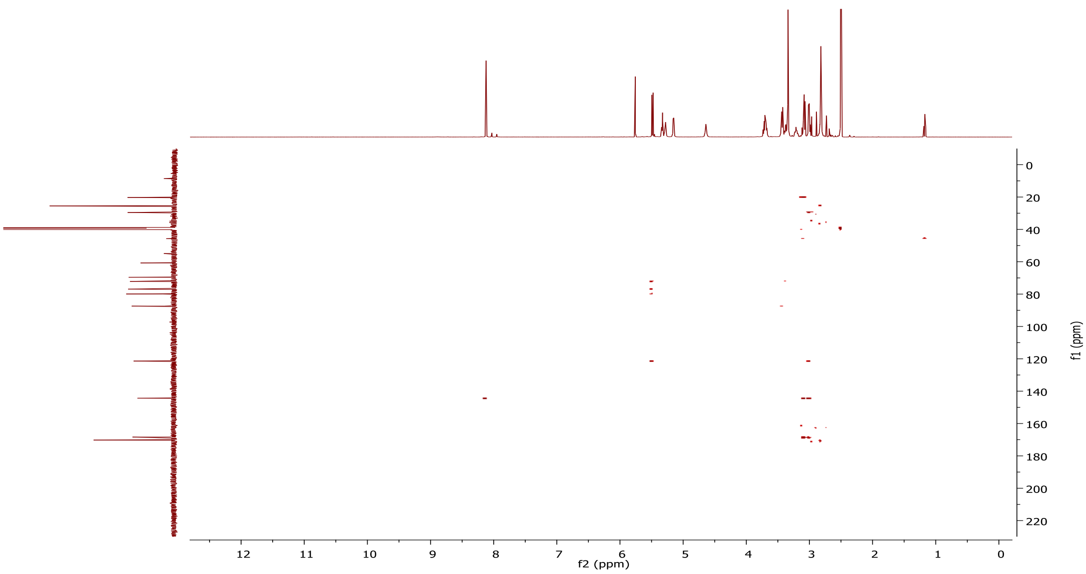

Figure S5e: HMBC NMR spectrum of **13** in DMSO

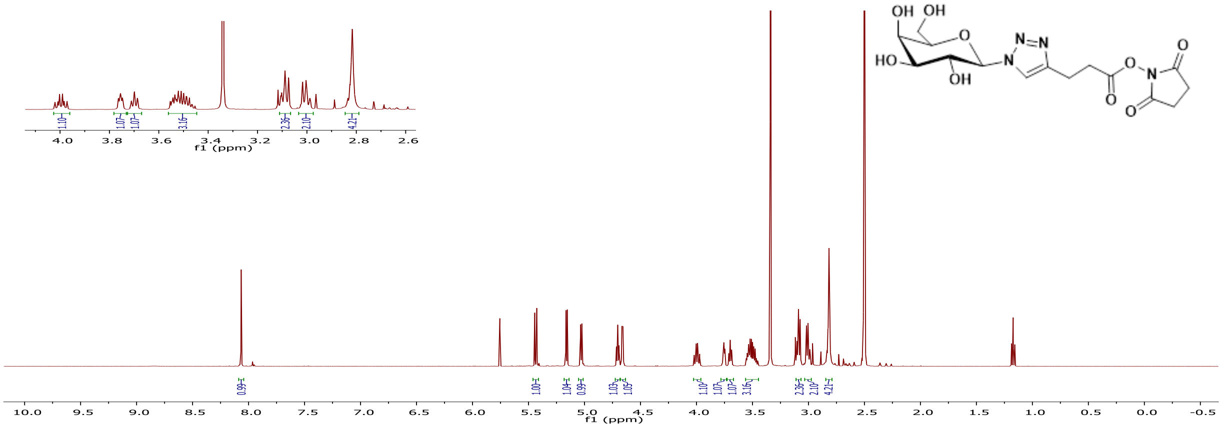

Figure S6a: ^{1}H NMR spectrum of **14** in DMSO

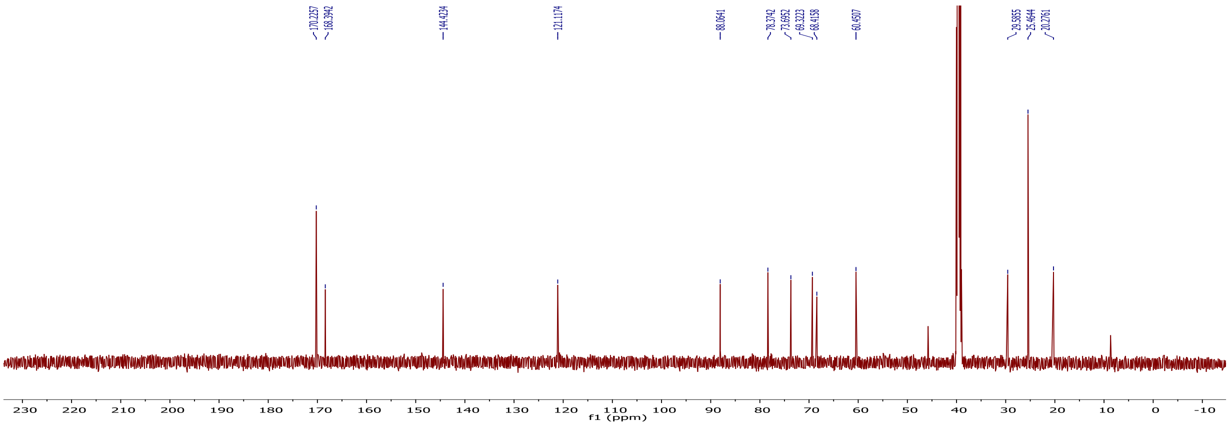

Figure S6b: ^{13}C NMR spectrum of **14** in DMSO

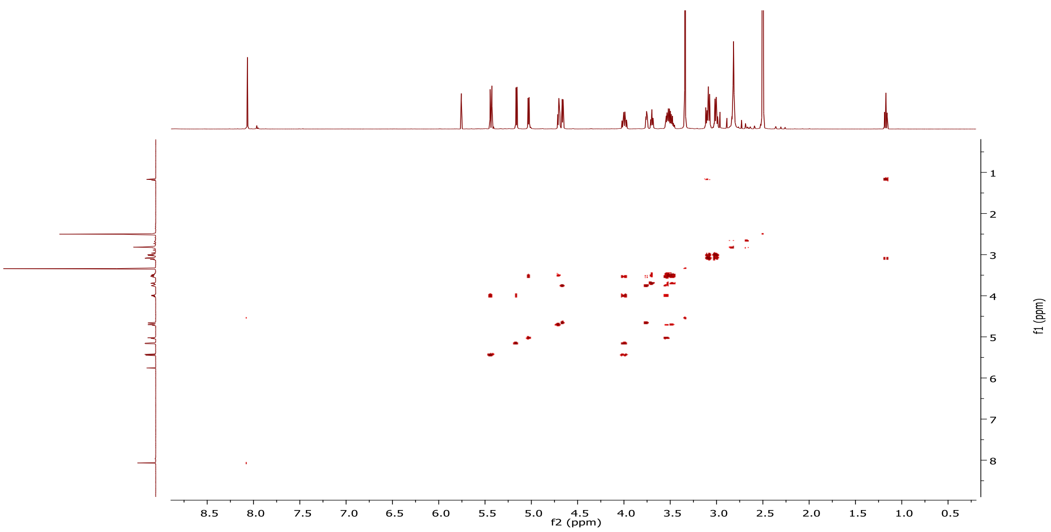

Figure S6c: COSY NMR spectrum of **14** in DMSO

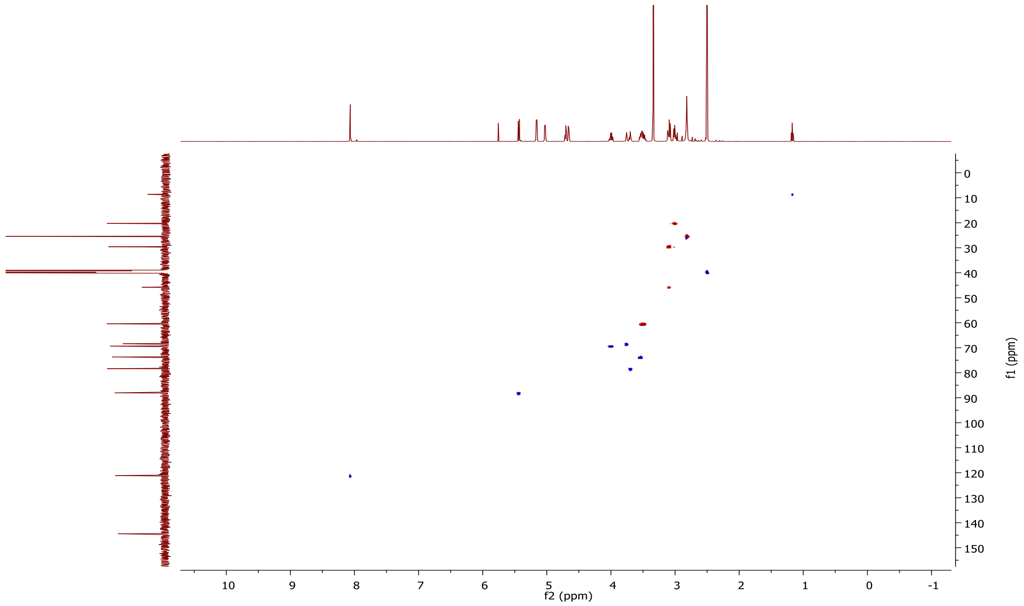

Figure S6d: HSQC NMR spectrum of **14** in DMSO

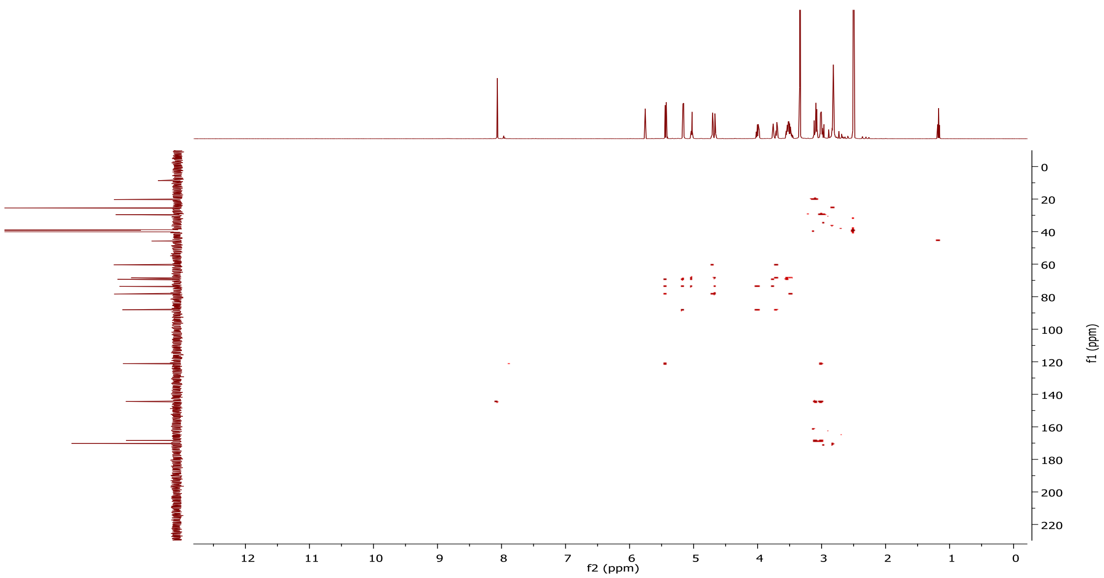

Figure S6e: HMBC NMR spectrum of **14** in DMSO

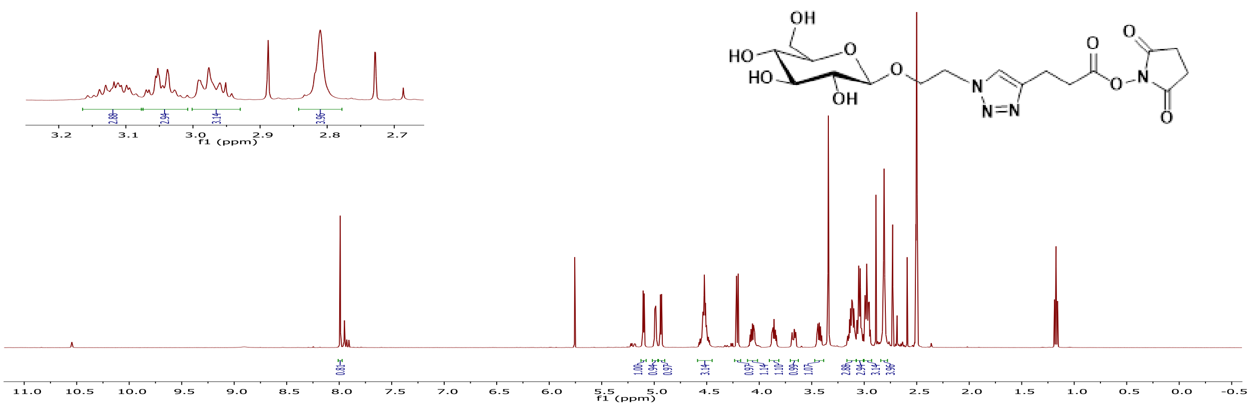

Figure S7a: ^{1}H NMR spectrum of **23** in DMSO

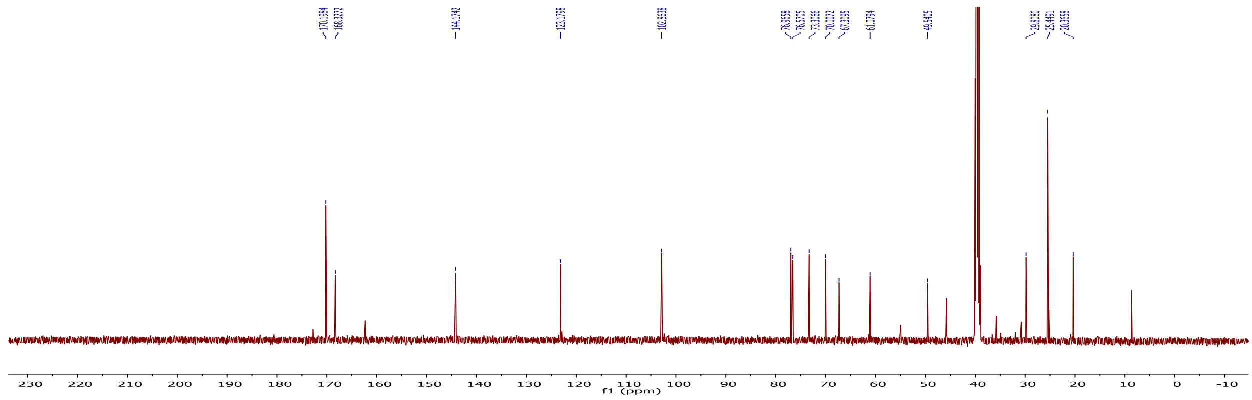

Figure S7b: ^{13}C NMR spectrum of **23** in DMSO

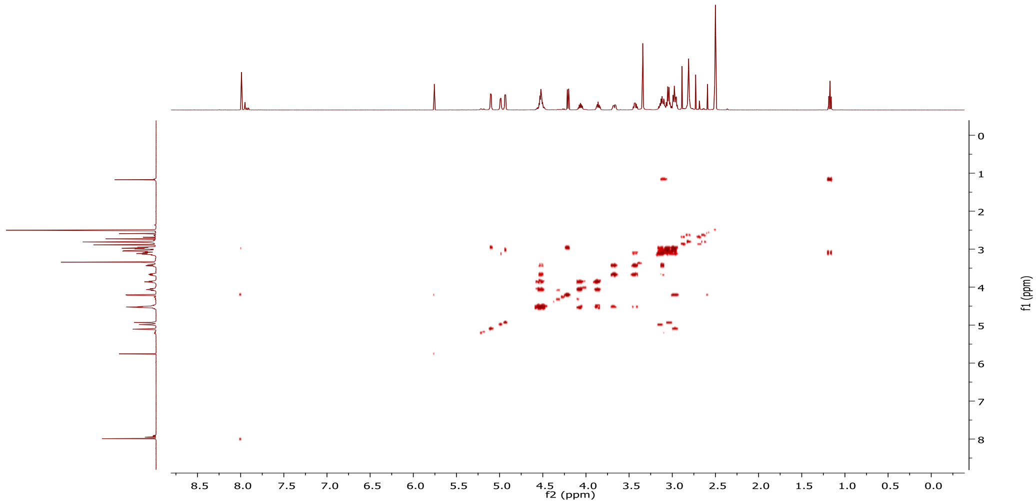

Figure S7c: COSY NMR spectrum of **23** in DMSO

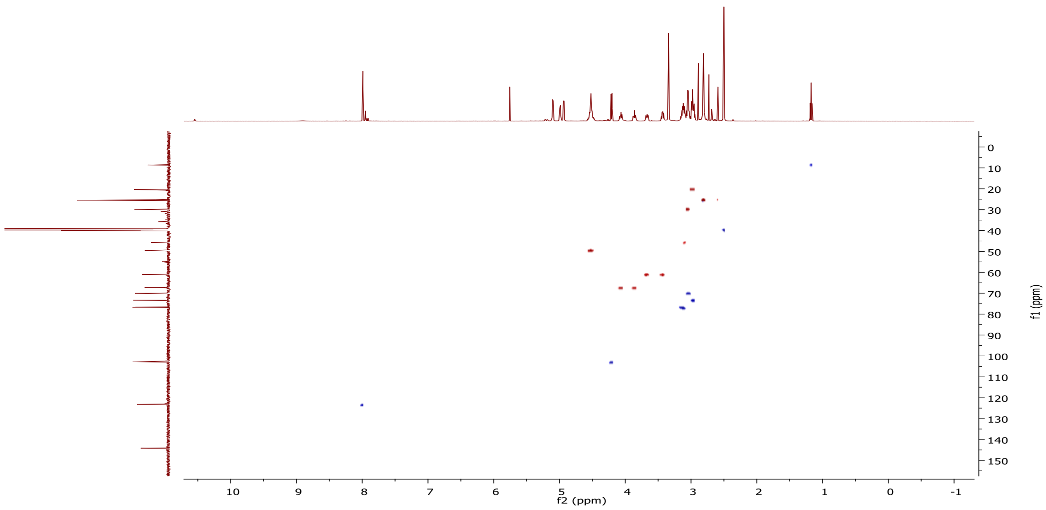

Figure S7d: HSQC NMR spectrum of **23** in DMSO

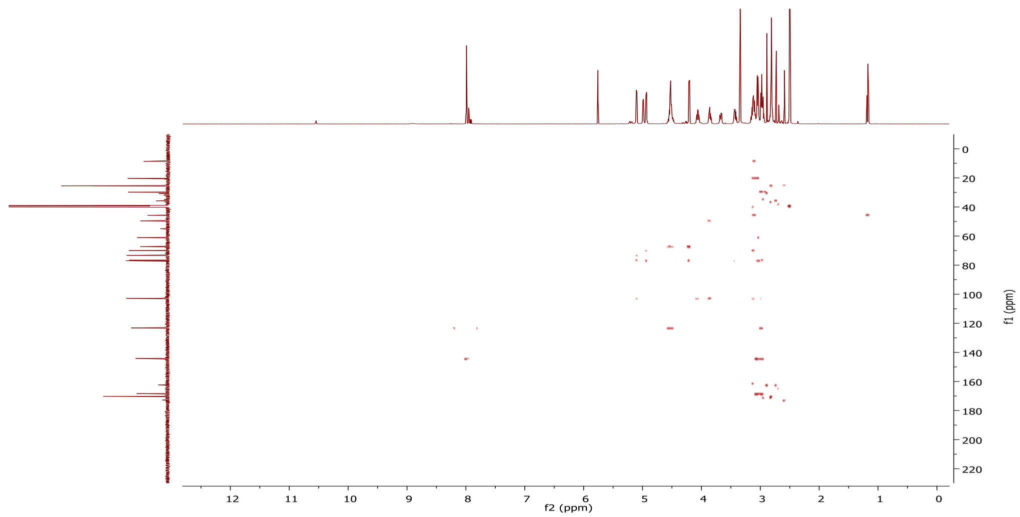

Figure S7e: HMBC NMR spectrum of **23** in DMSO

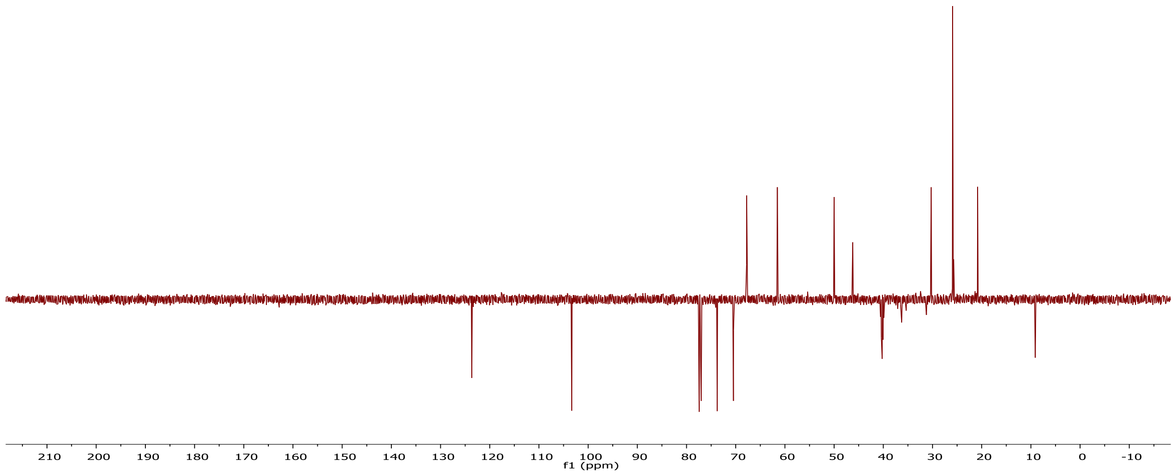

Figure S7f: DEPT-135 NMR spectrum of **23** in DMSO

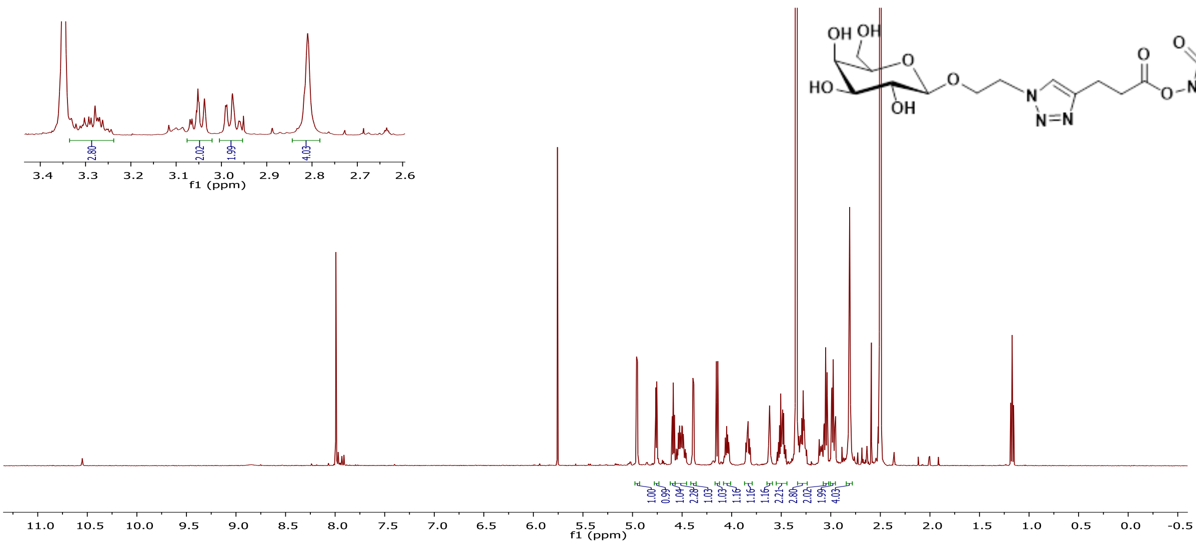

Figure S8a: ^{1}H NMR spectrum of **24** in DMSO

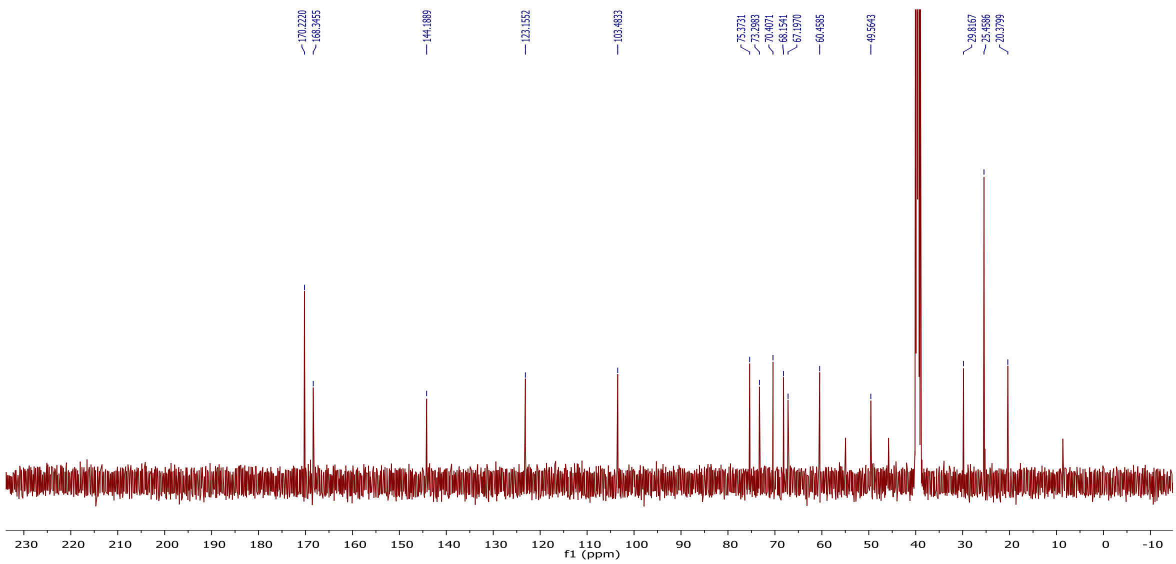

Figure S8b: ^{13}C NMR spectrum of **24** in DMSO

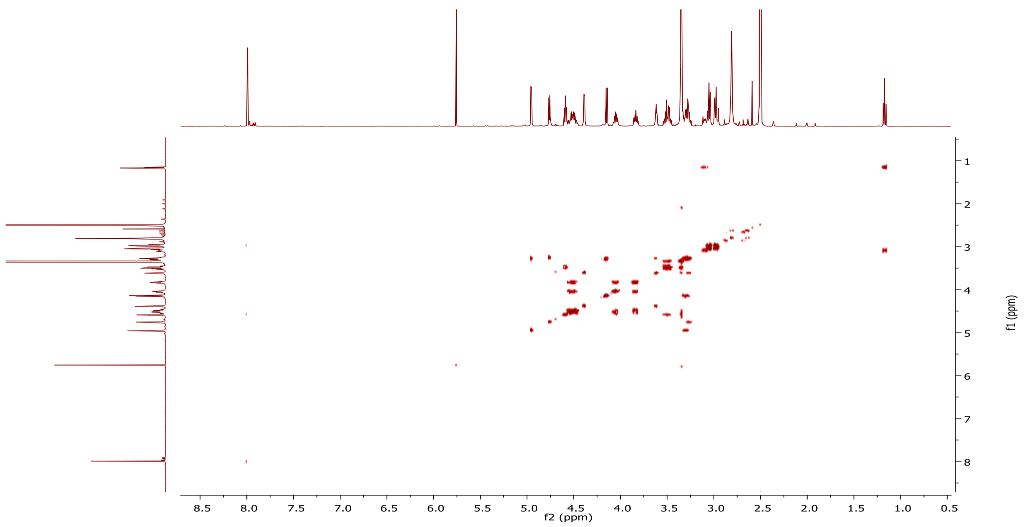

Figure S8c: COSY NMR spectrum of **24** in DMSO

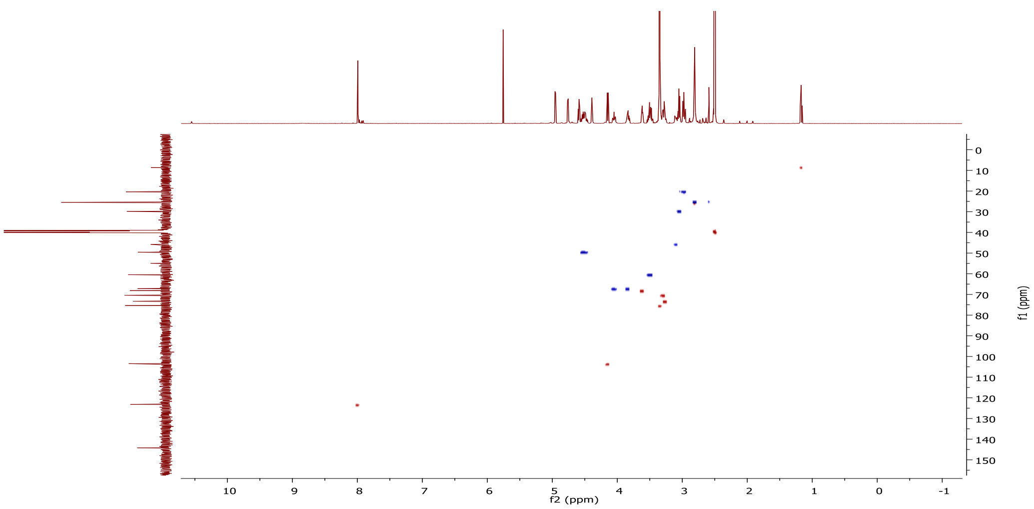

Figure S8d: HSQC NMR spectrum of **24** in DMSO

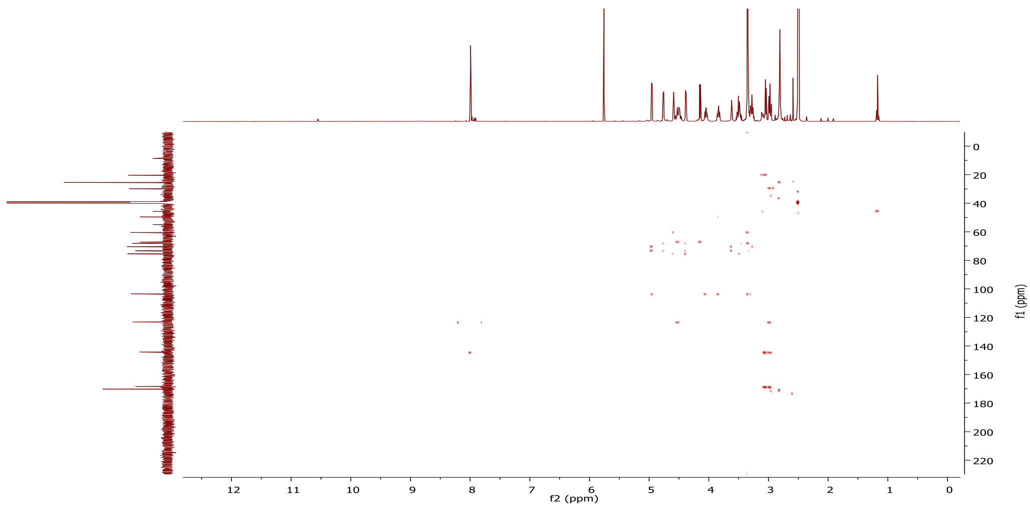

Figure S8e: HMBC NMR spectrum of **24** in DMSO

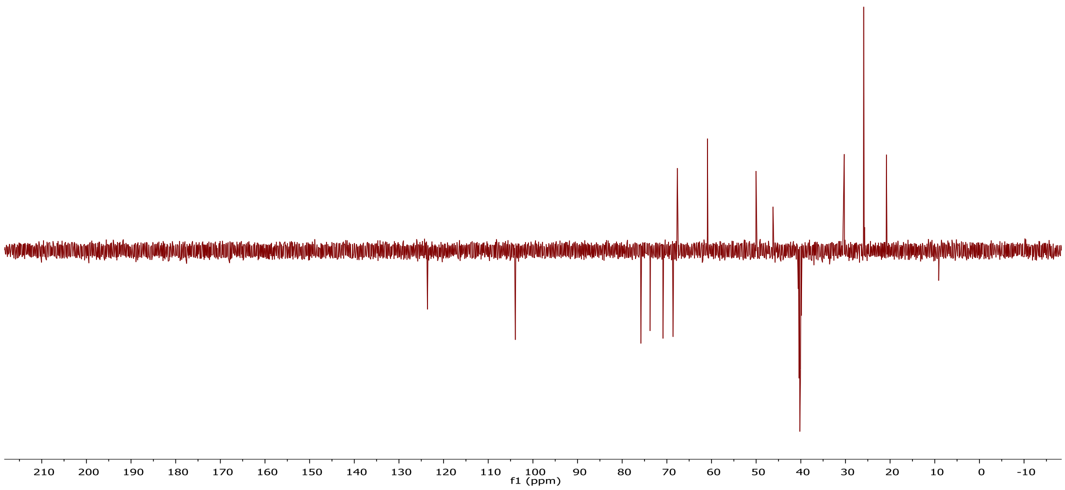

Figure S8f: DEPT-135 NMR spectrum of **24** in DMSO

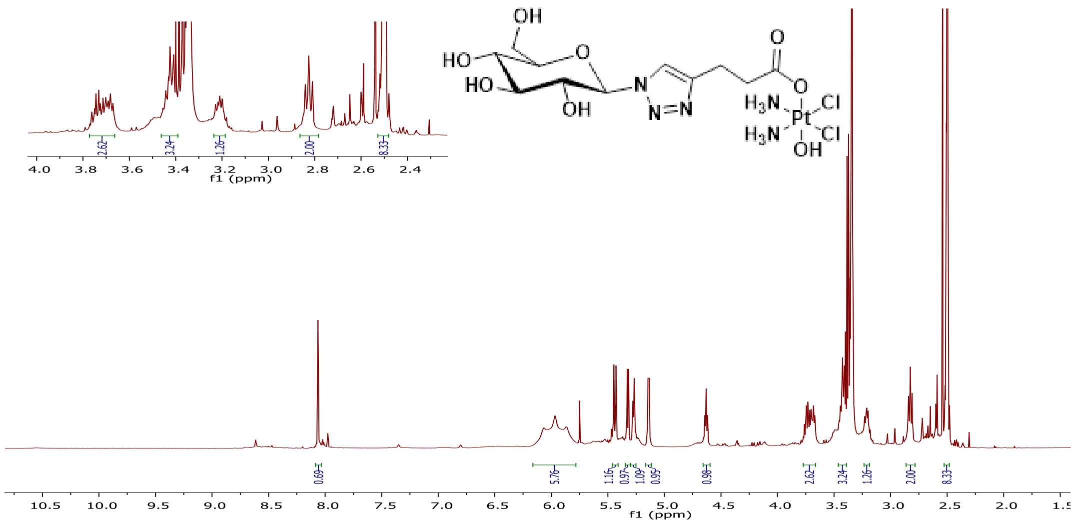

Figure S9a: ^{1}H NMR spectrum of **1** in DMSO

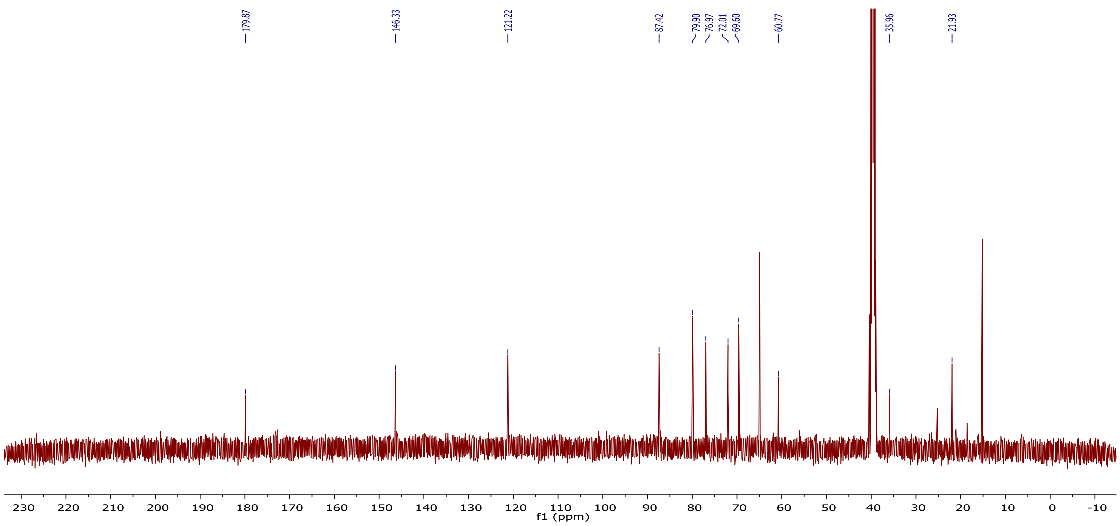

Figure S9b: ^{13}C NMR spectrum of **1** in DMSO

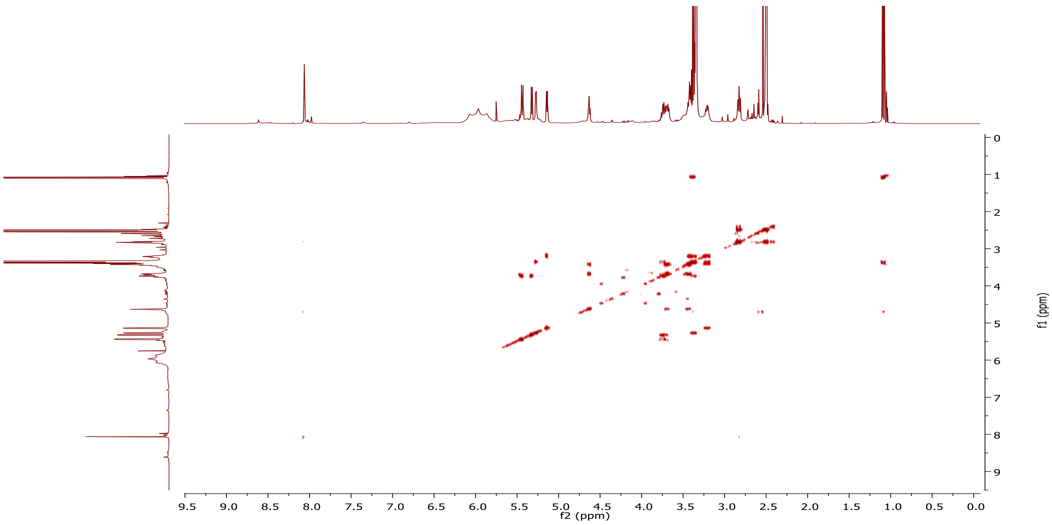

Figure S9c: COSY NMR spectrum of **1** in DMSO

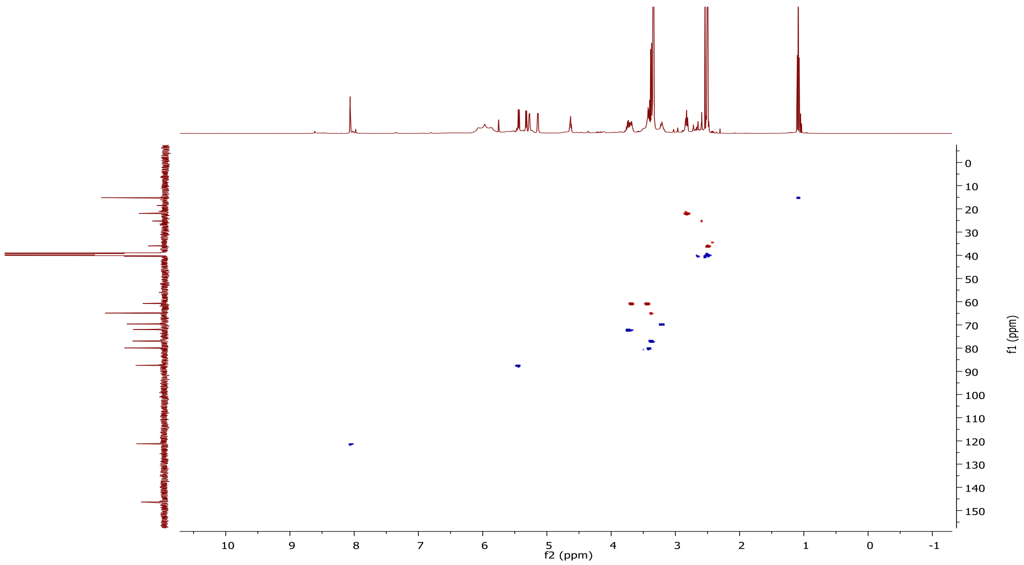

Figure S9d: HSQC NMR spectrum of **1** in DMSO

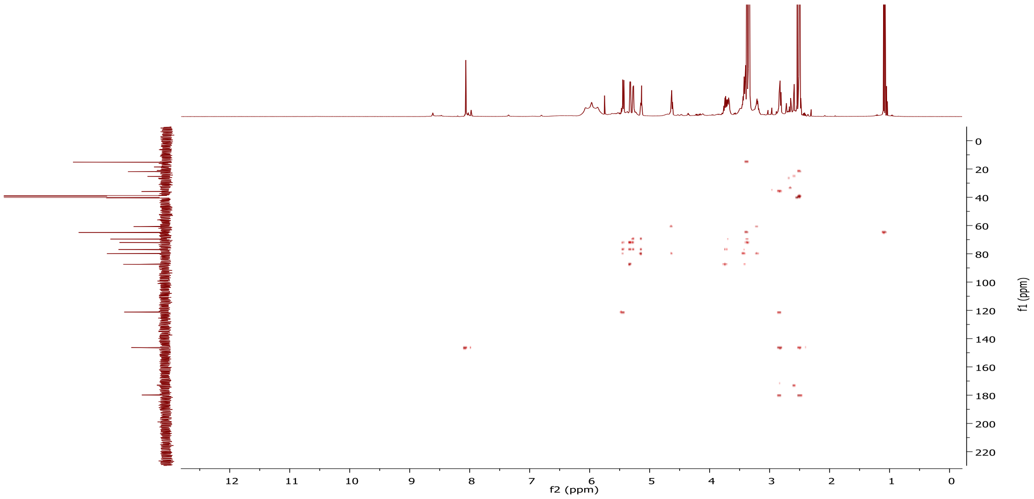

Figure S9e: HMBC NMR spectrum of **1** in DMSO

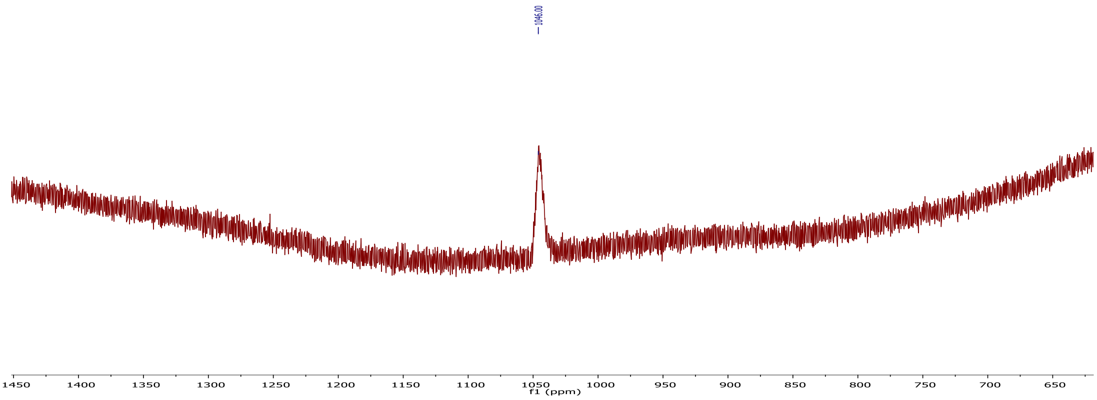

Figure S9f: ^{195}Pt NMR spectrum of **1** in DMSO

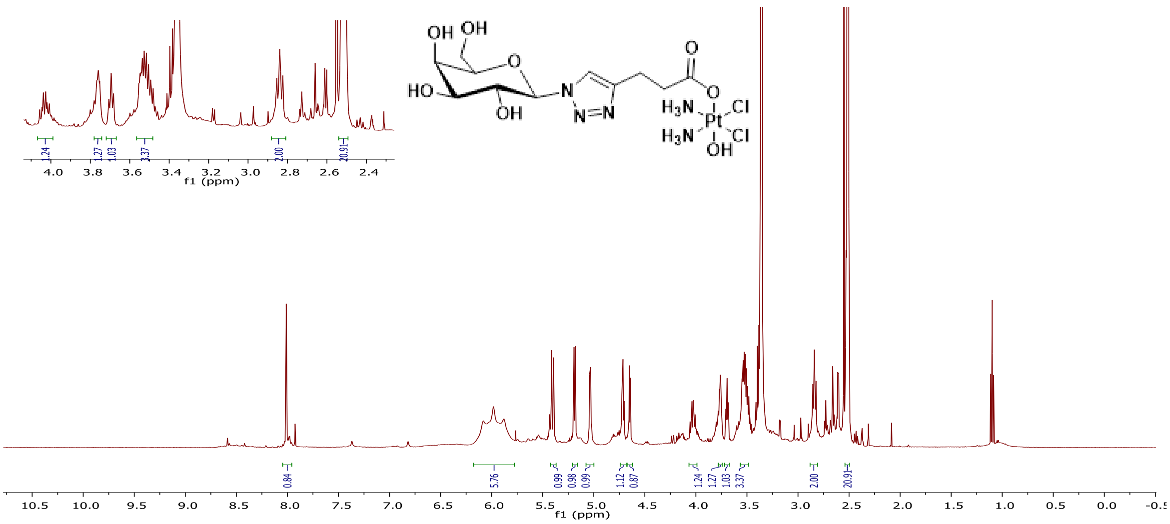

Figure S10a: ^{1}H NMR spectrum of **2** in DMSO

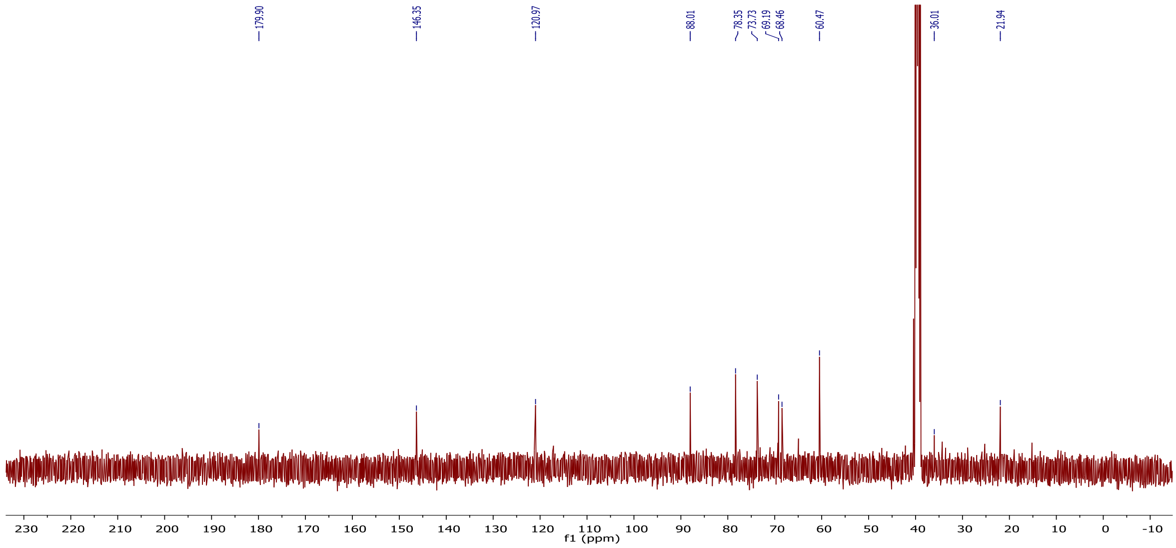

Figure S10b: ^{13}C NMR spectrum of **2** in DMSO

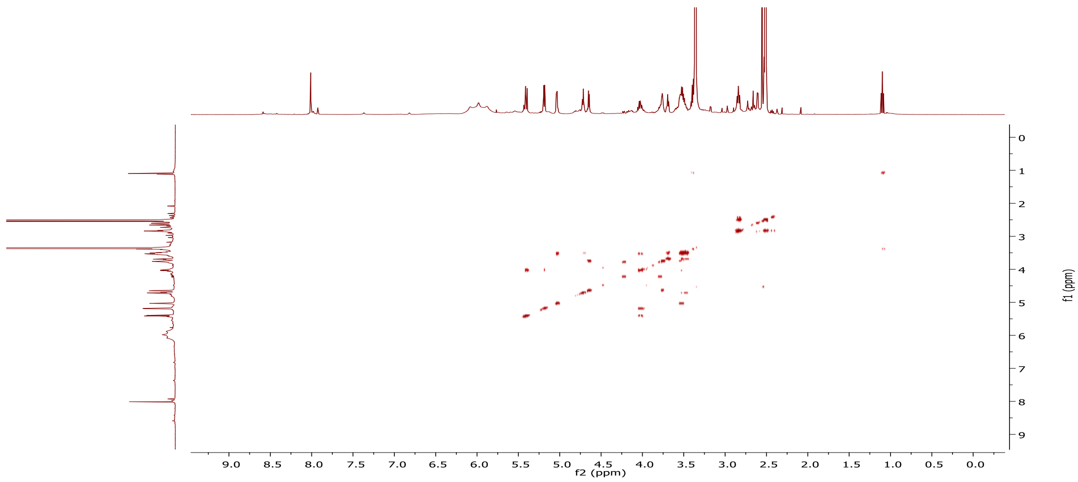

Figure S10c: COSY NMR spectrum of **2** in DMSO

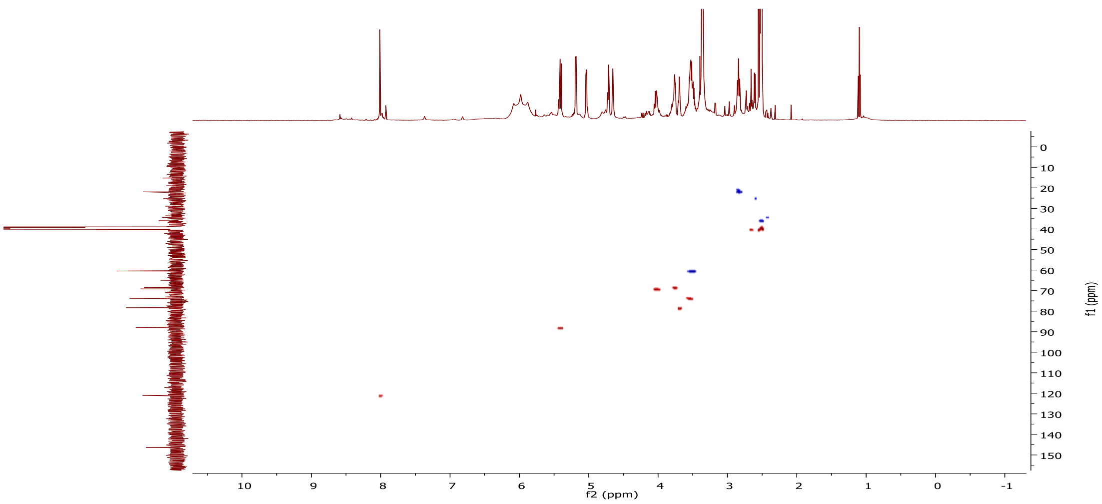

Figure S10d: HSQC NMR spectrum of **2** in DMSO

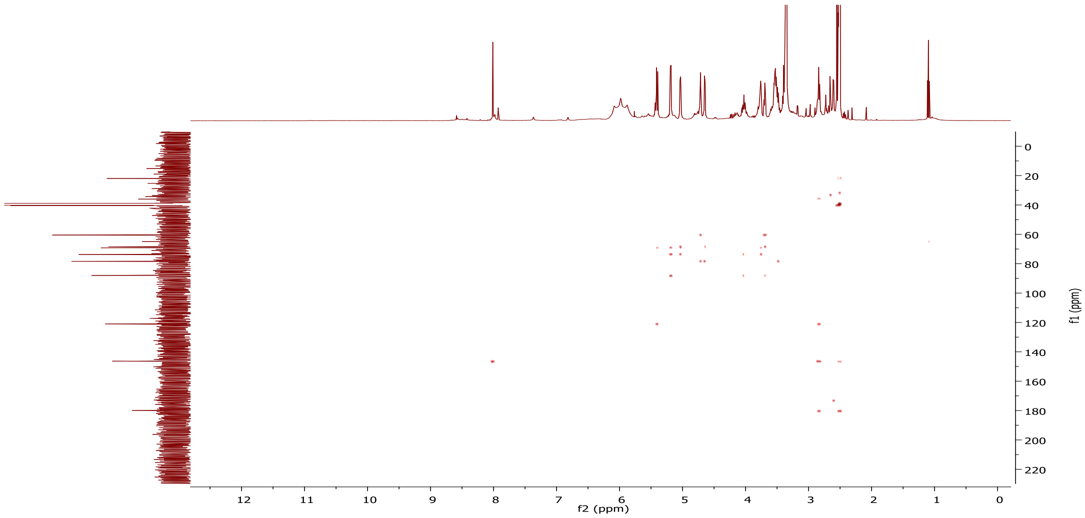

Figure S10e: HMBC NMR spectrum of **2** in DMSO

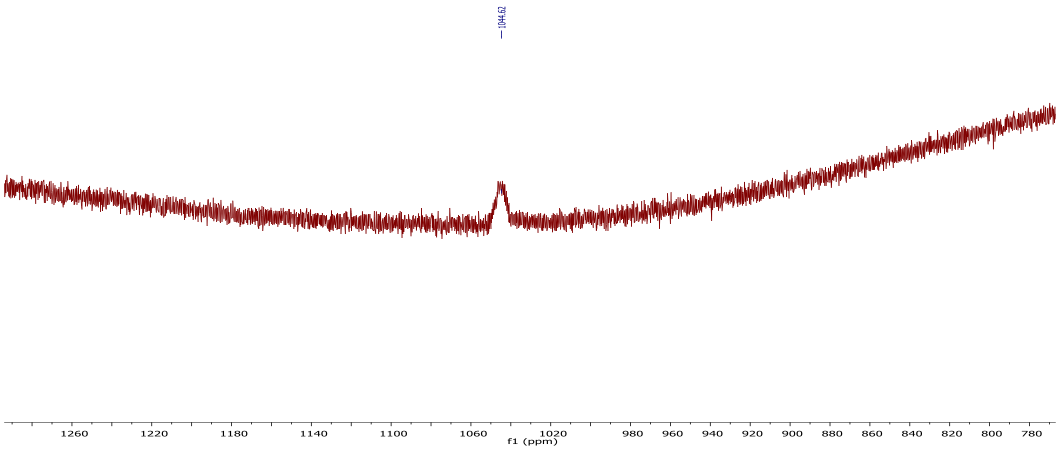

Figure S10f: ^{195}Pt NMR spectrum of **2** in DMSO

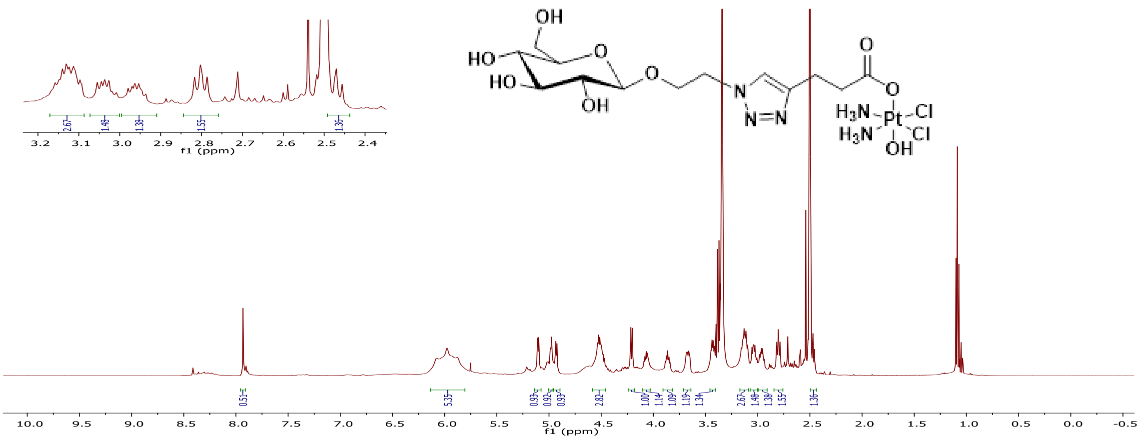

Figure S11a: ^{1}H NMR spectrum of **3** in DMSO

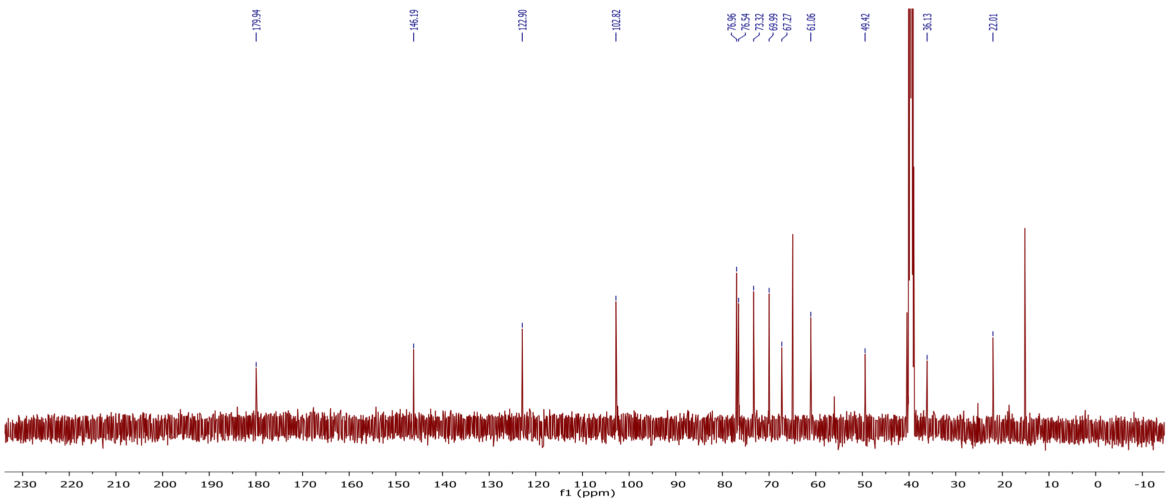

Figure S11b: ^{13}C NMR spectrum of **3** in DMSO

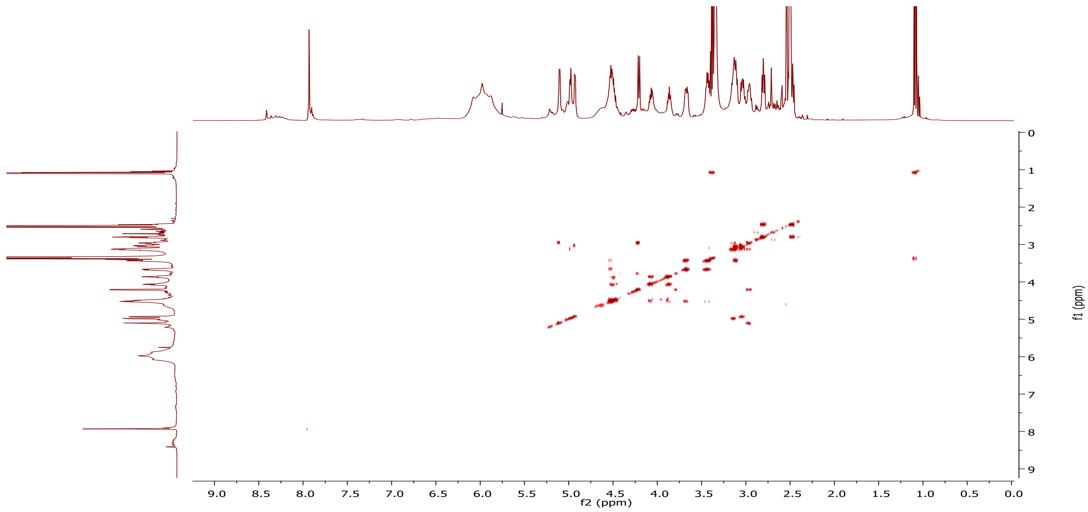

Figure S11c: COSY NMR spectrum of **3** in DMSO

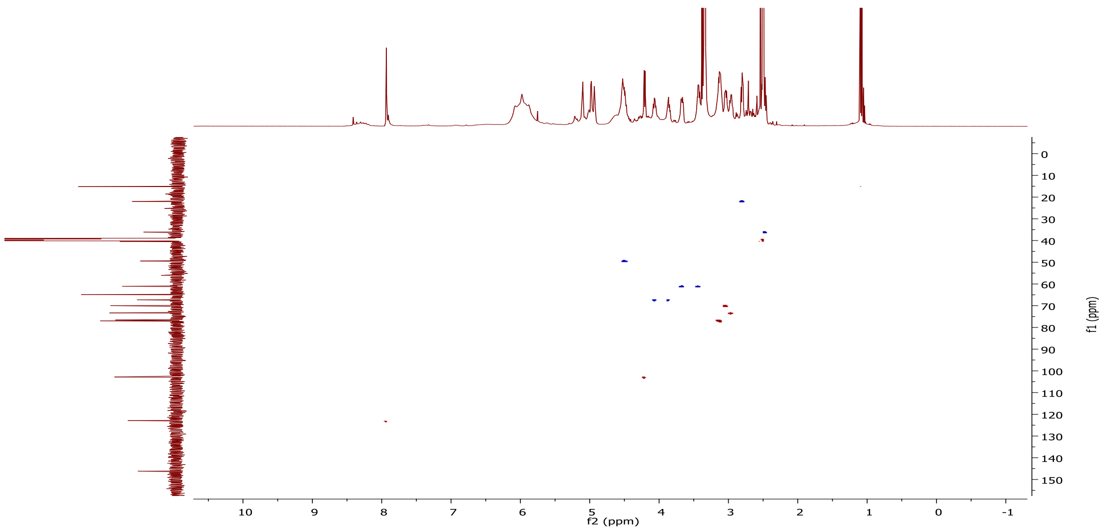

Figure S11d: HSQC NMR spectrum of **3** in DMSO

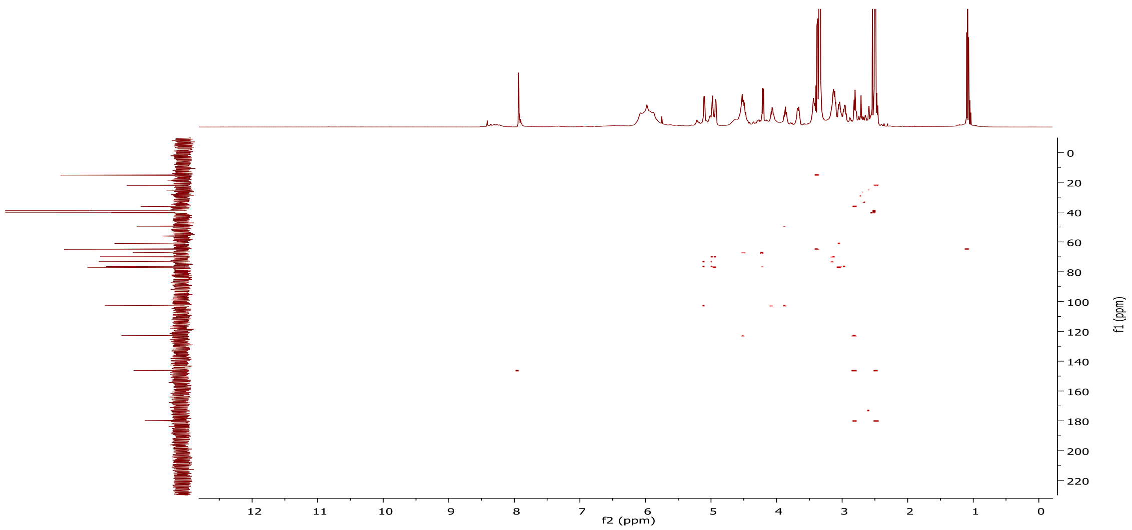

Figure S11e: HMBC NMR spectrum of **3** in DMSO

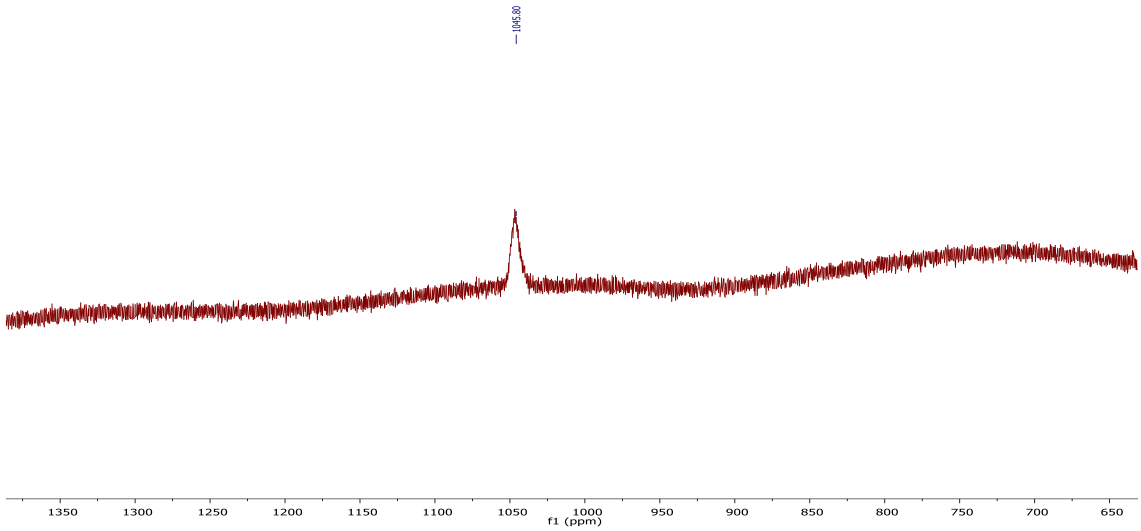

Figure S11f: ^{195}Pt NMR spectrum of **3** in DMSO

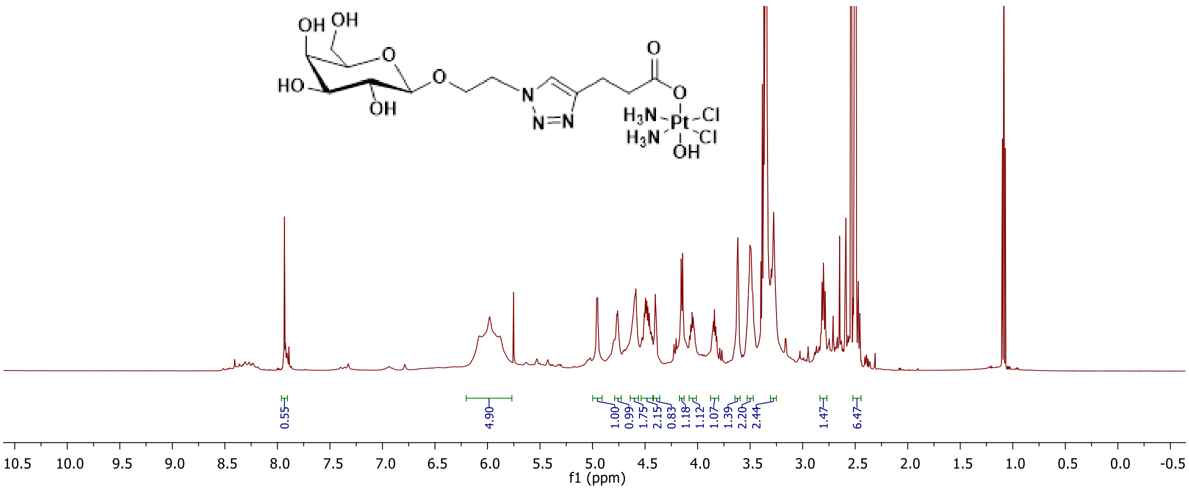

Figure S12a: ^{1}H NMR spectrum of **4** in DMSO

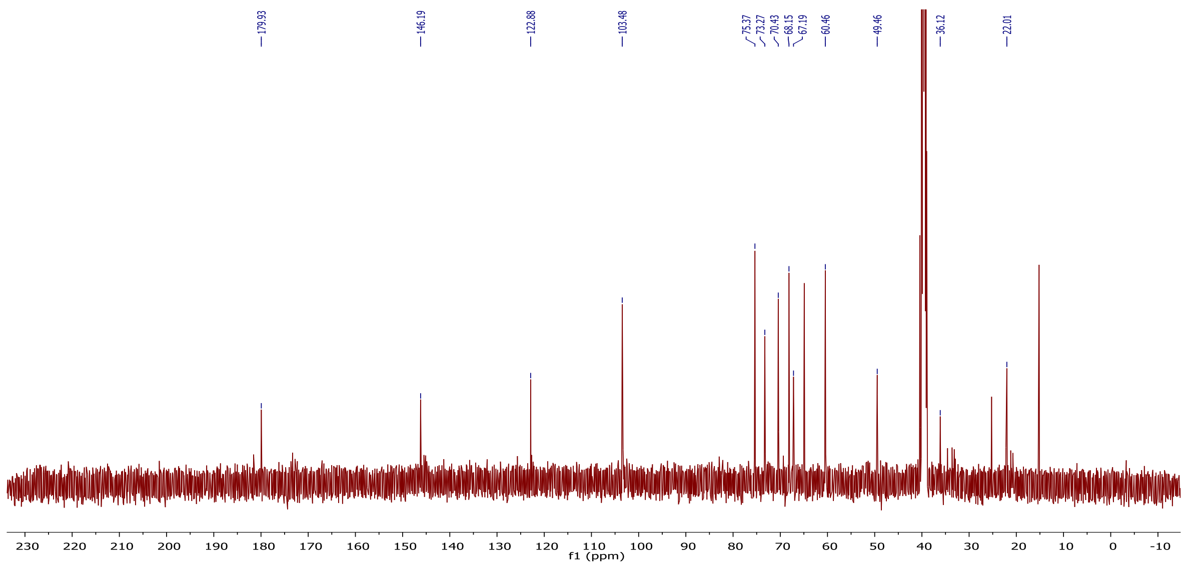

Figure S12b: ^{13}C NMR spectrum of **4** in DMSO

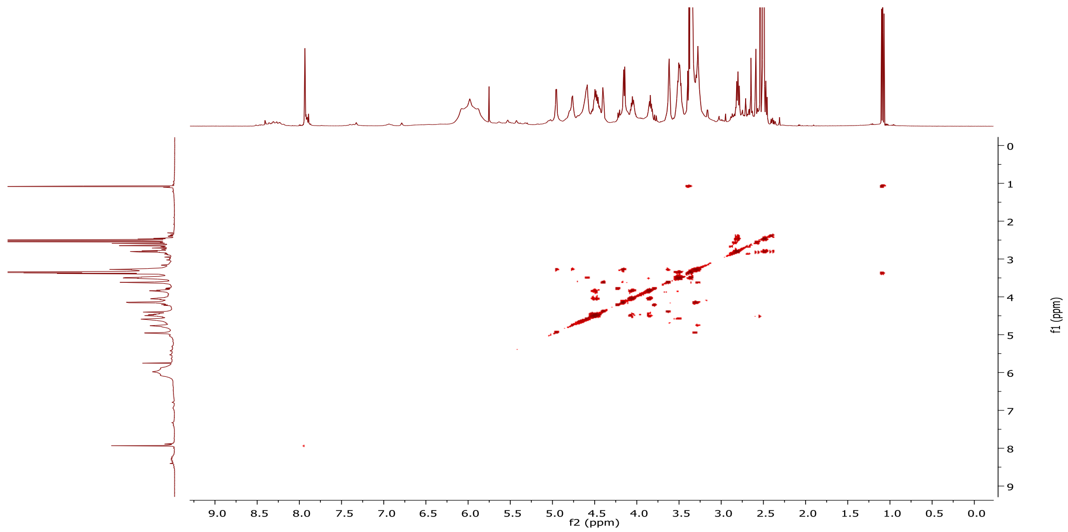

Figure S12c: COSY NMR spectrum of **4** in DMSO

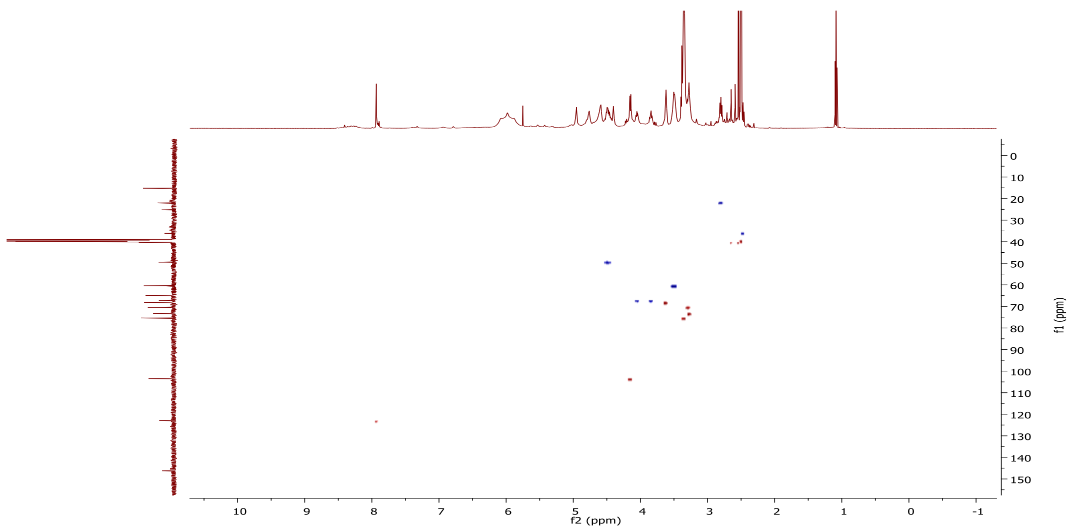

Figure S12d: HSQC NMR spectrum of **4** in DMSO

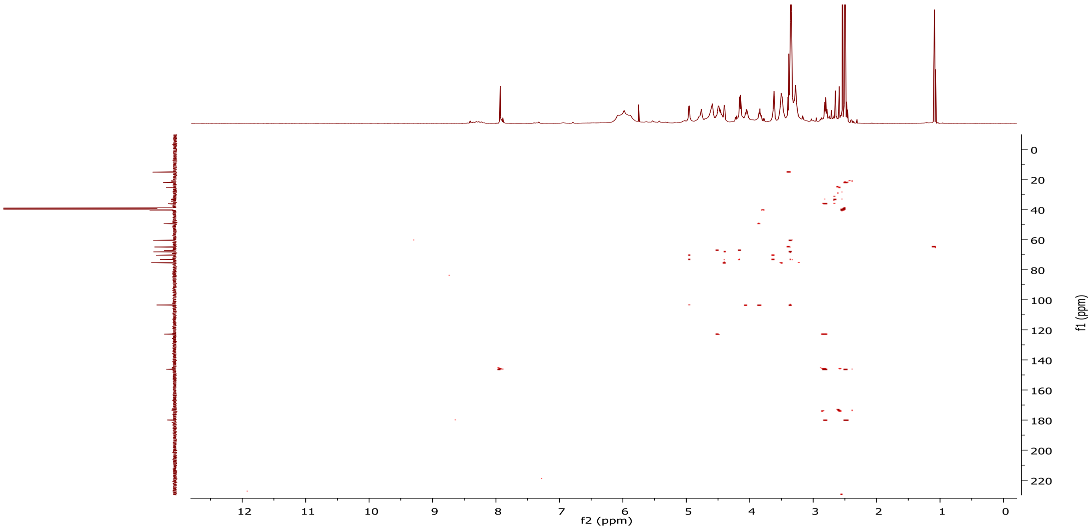

Figure S12e: HMBC NMR spectrum of **4** in DMSO

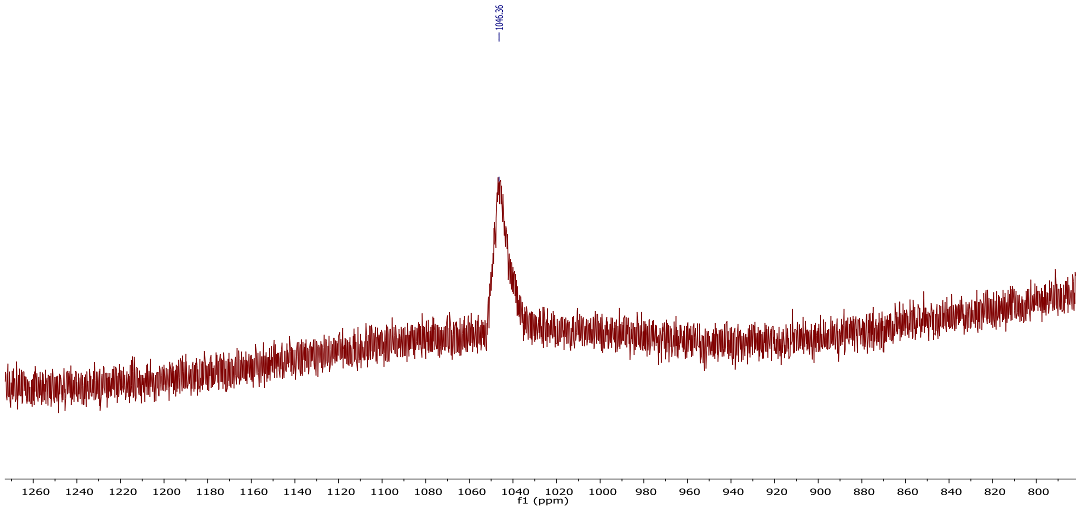

Figure S12f: ^{195}Pt NMR spectrum of **4** in DMSO

HR-MS Spectra

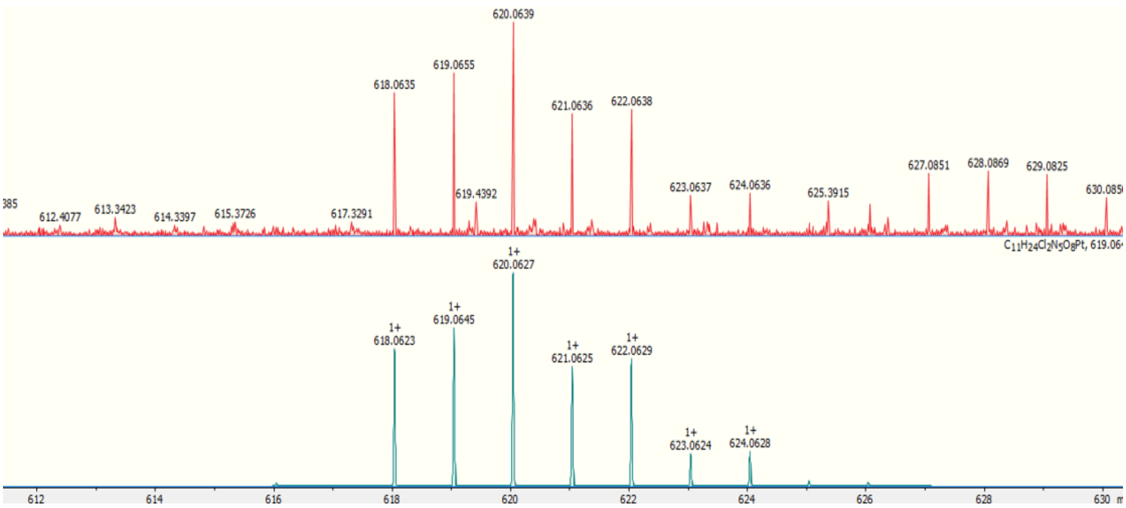

Figure S13: HR-MS spectrum of **1**

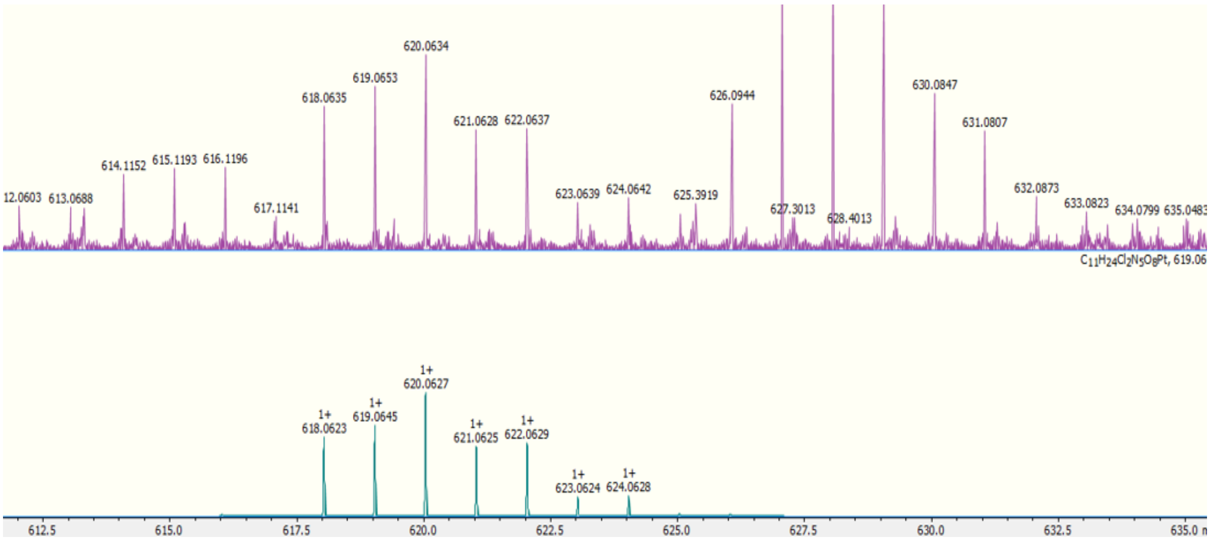

Figure S14: HR-MS spectrum of **2**

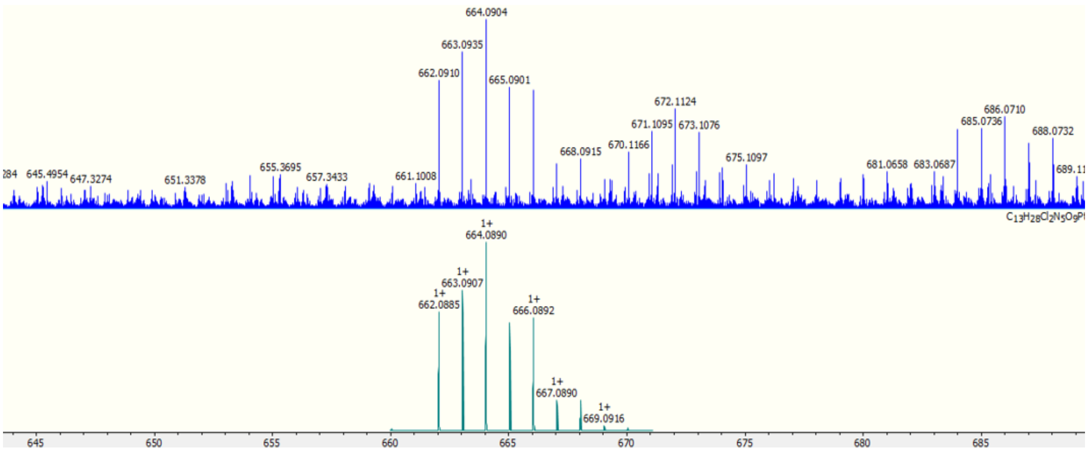

Figure S15: HR-MS spectrum of **3**

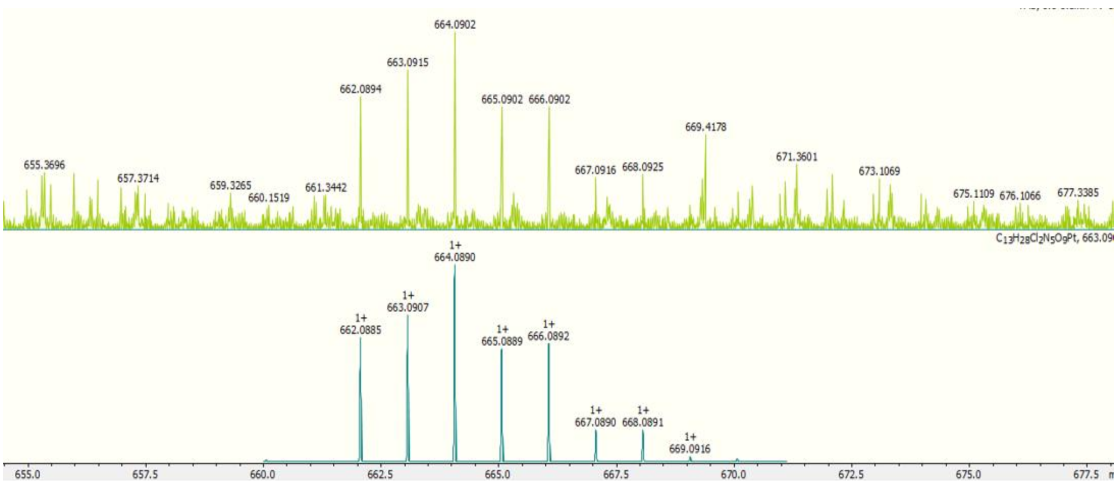

Figure S16: HR-MS spectrum of **4**

Stability

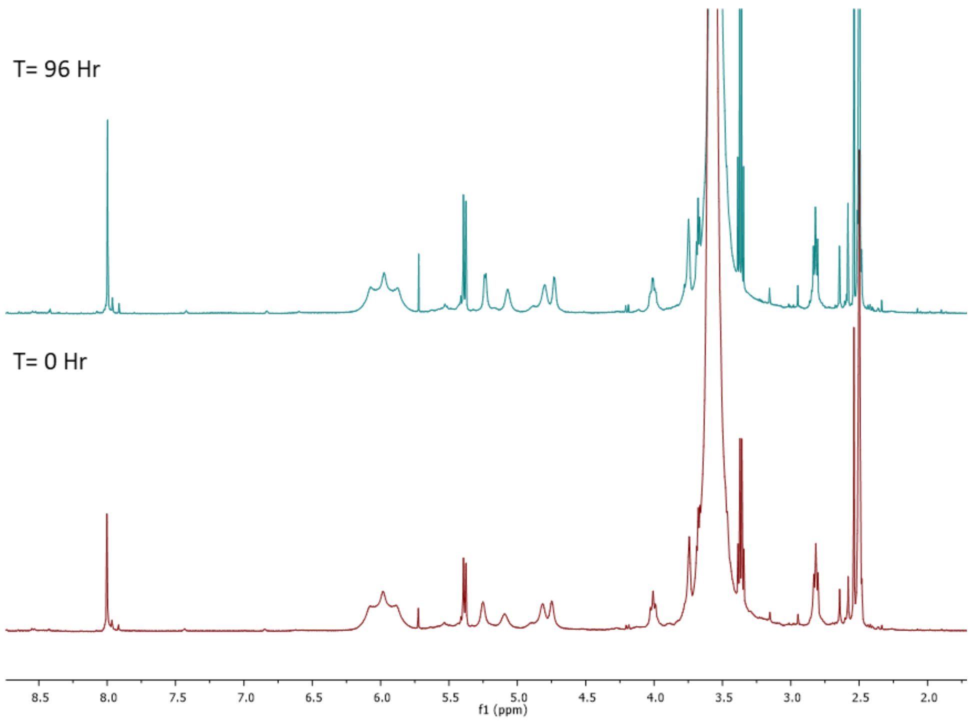

Figure S17a: ^{1}H NMR of **1** in DMSO and PBS buffer at 0 and 96 hours.

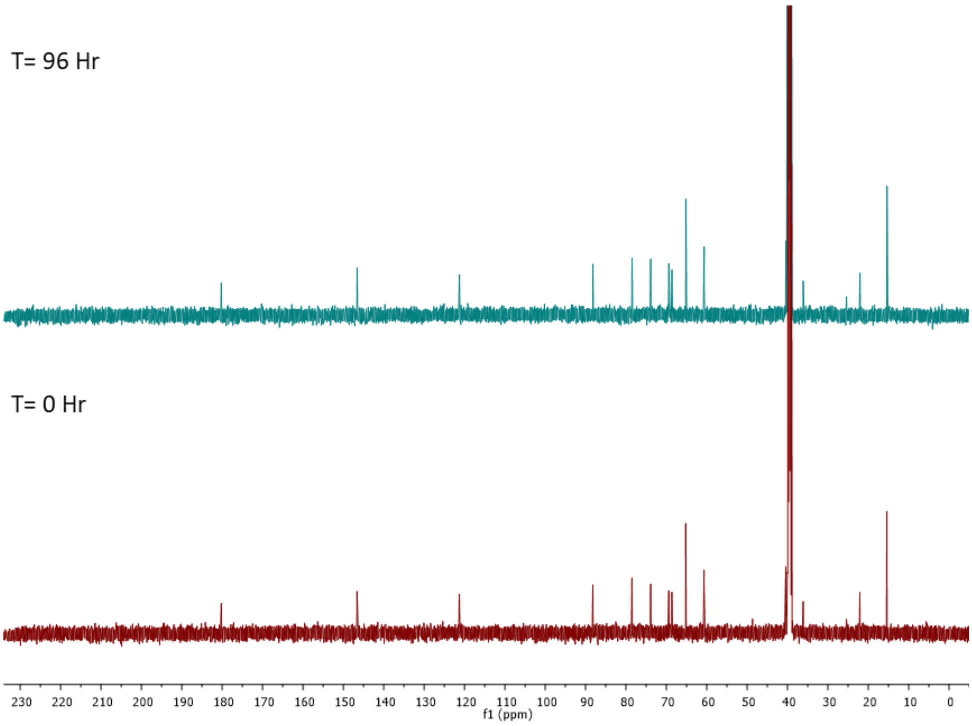

Figure S17b: ^{13}C NMR of **1** in DMSO and PBS buffer at 0 and 96 hours.

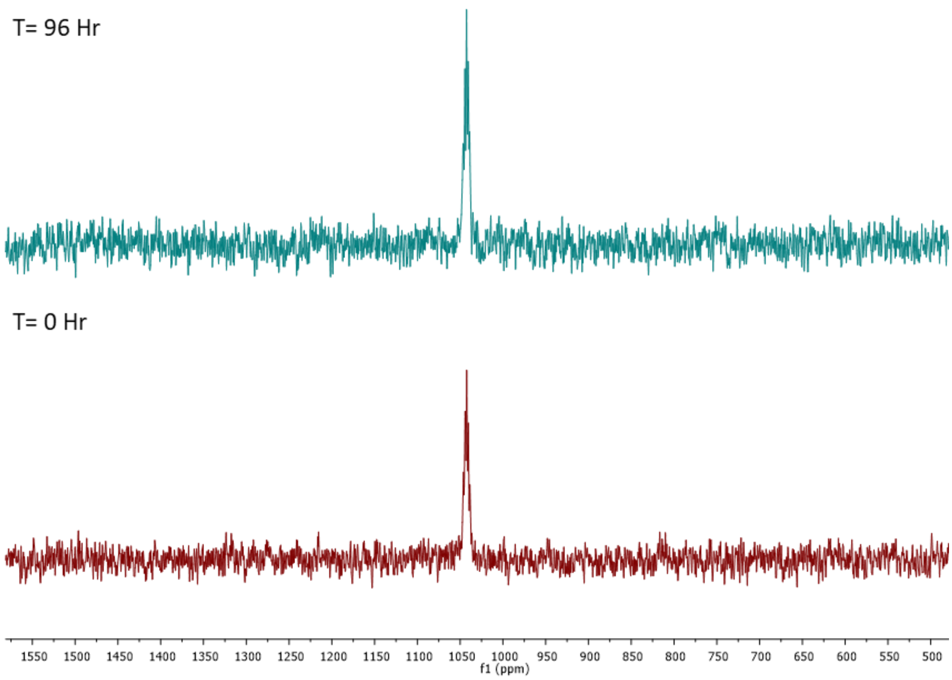

Figure S17c: ^{195}Pt NMR of **1** in DMSO and PBS buffer at 0 and 96 hours

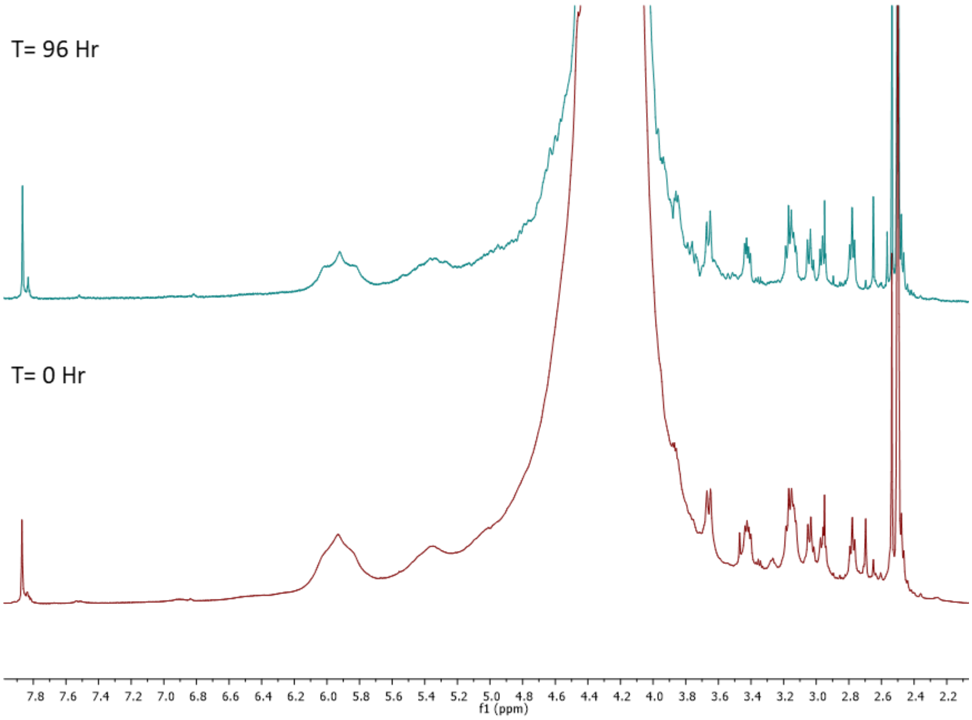

Figure S17d: ^{1}H NMR of **3** in DMSO and PBS buffer at 0 and 96 hours

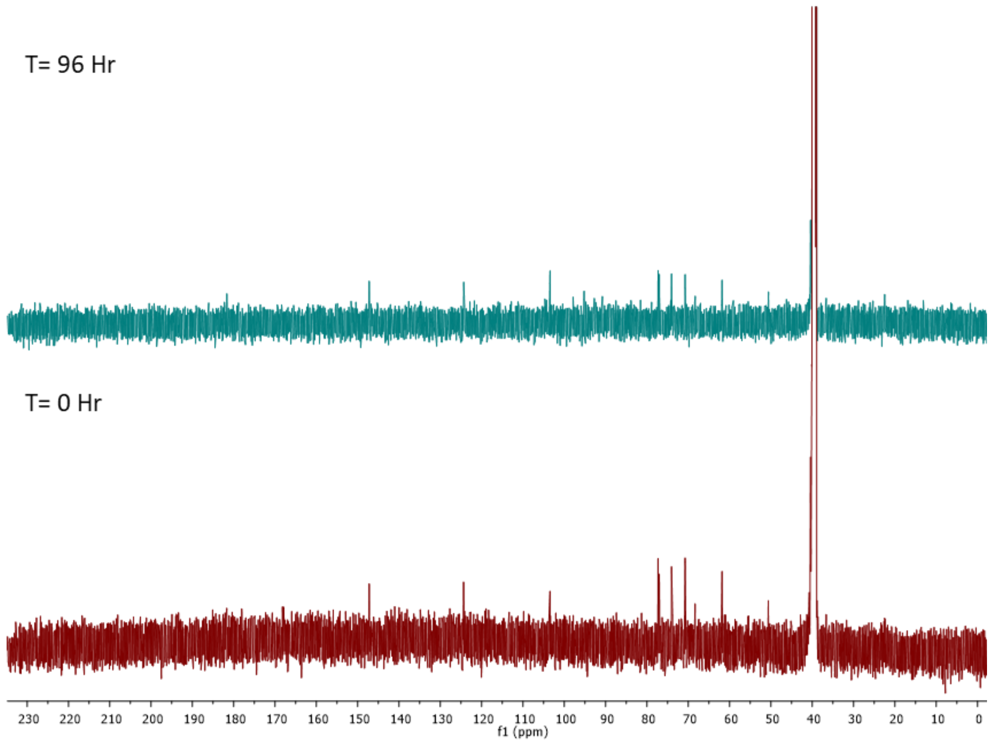

Figure S17e: ^{13}C NMR of **3** in DMSO and PBS buffer at 0 and 96 hours.

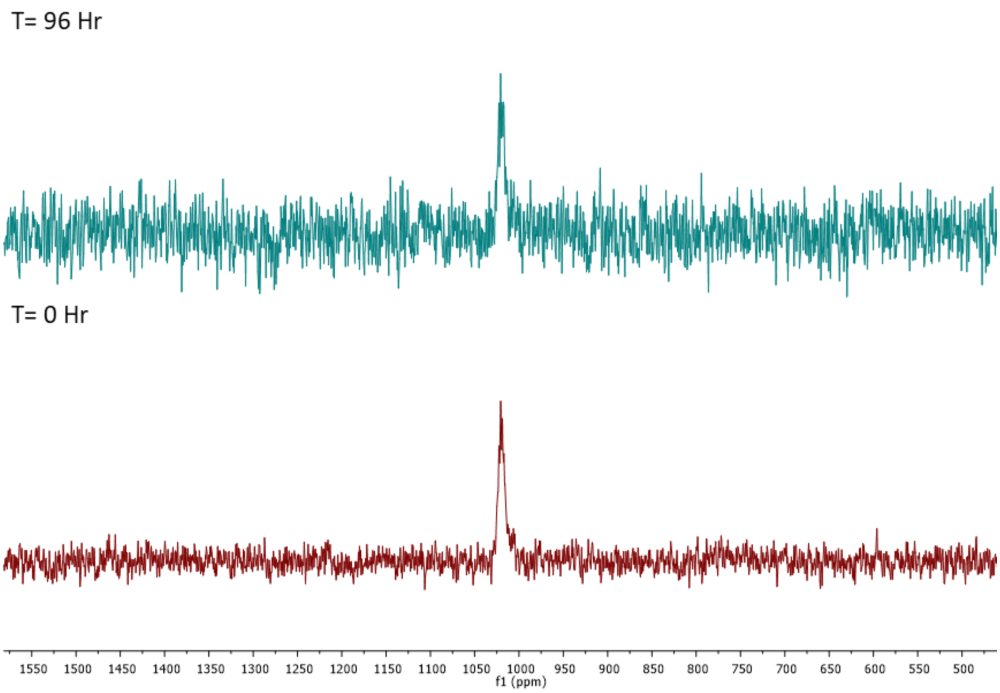

Figure S17f: ^{195}Pt NMR of **3** in DMSO and PBS at 0 and 96 hours.

Cyclic Voltammetry

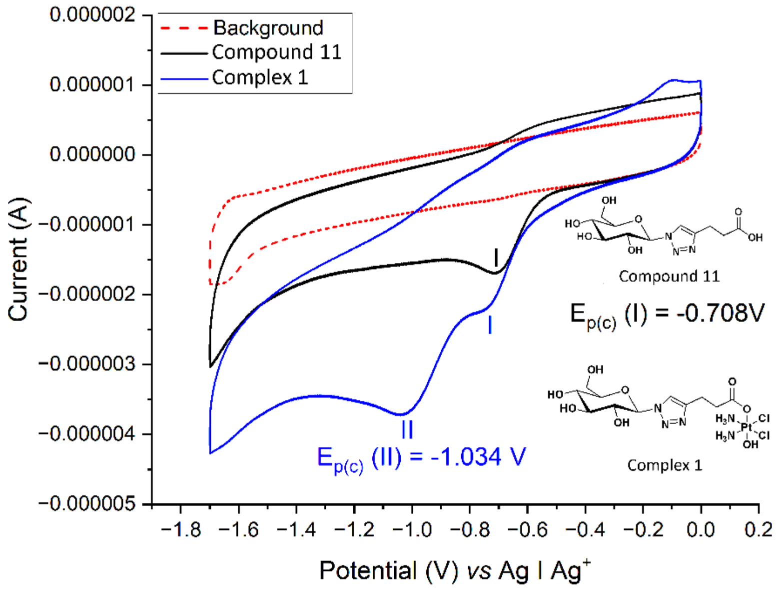

Figure S18: Cyclic voltammogram of Complex **1** and ligand **11**

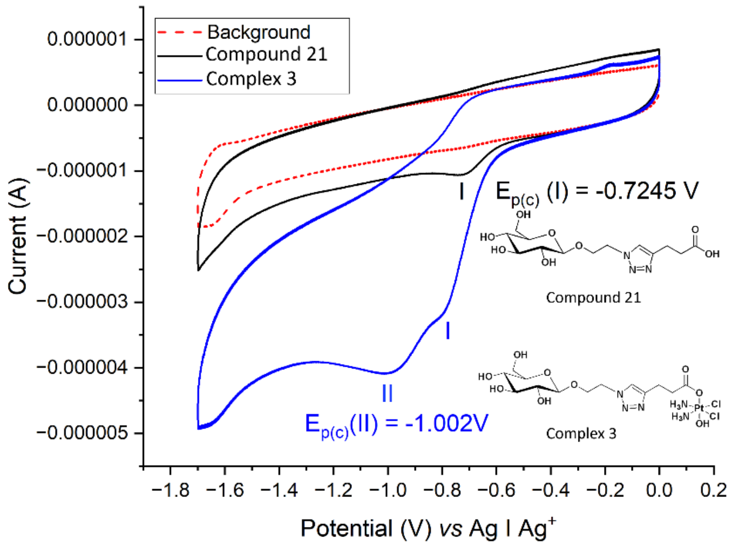

Figure S19: Cyclic voltammogram of Complex **3** and ligand **21**

Reduction Study

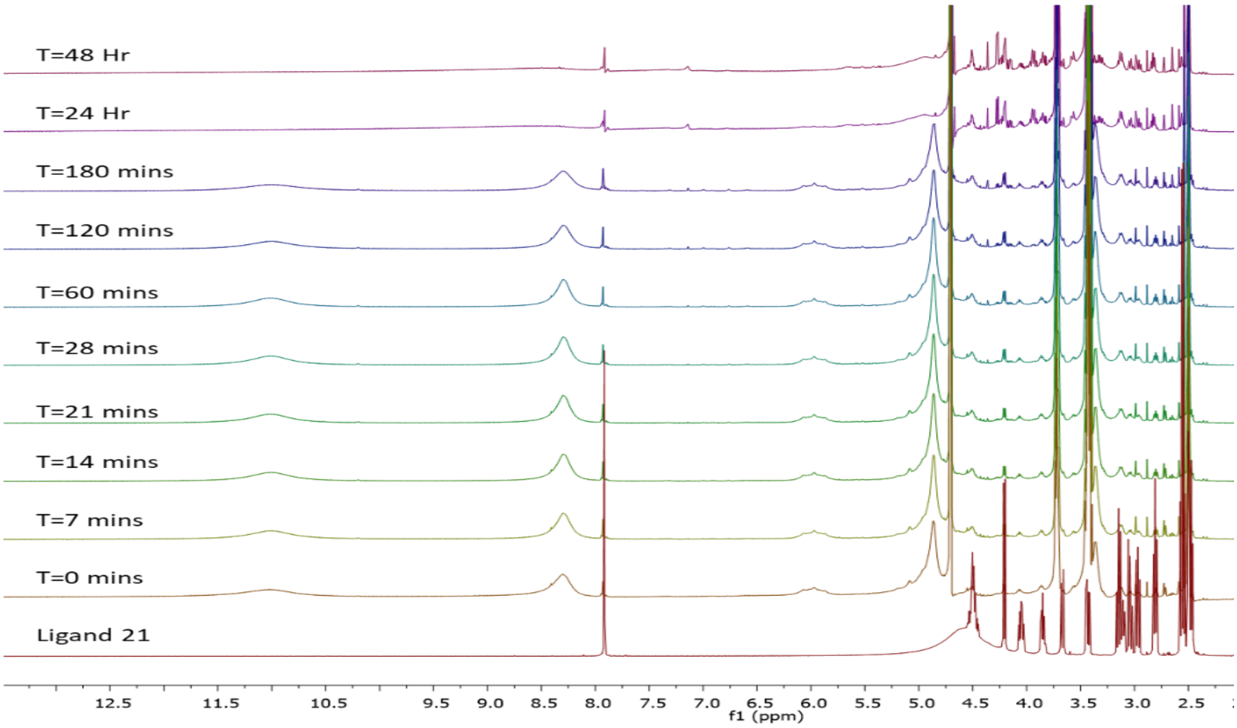

Figure S20: [1]H-NMR spectra of the complex **3** with addition of 10 eq. of ascorbic acid. NMR spectra were collected every 7minutes for 30 minutes and then every hour for 3 hours and finally left to reduce for 2 days.

Biological Figures

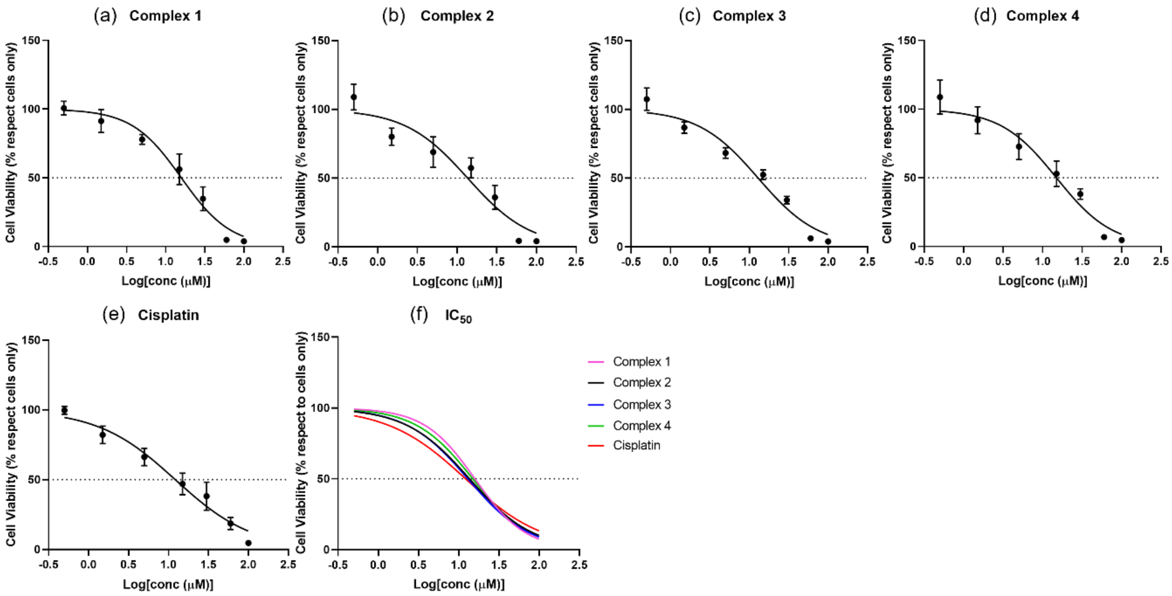

Figure S21: 2D *in vitro* screening of complexes 1-4 on MG63 cell line by MTT assay and IC_{50} (μM) values. Dose-response curves used to generate IC_{50} (μM) for complexes 1 (a), 2 (b), 3 (c) and 4 (d), and for cisplatin (e) inhibitor activity on cell viability of MG63 cell line. The Log[concentration] in μM and the normalized response (%) of survival fraction of cells are reported on X and Y asses, respectively. For each complex, the curve interpolation with 50% survival cells is highlighted in Y dotted line and correspond to its $LogIC_{50}$. A comparison of all dose-response curves is reported (f).

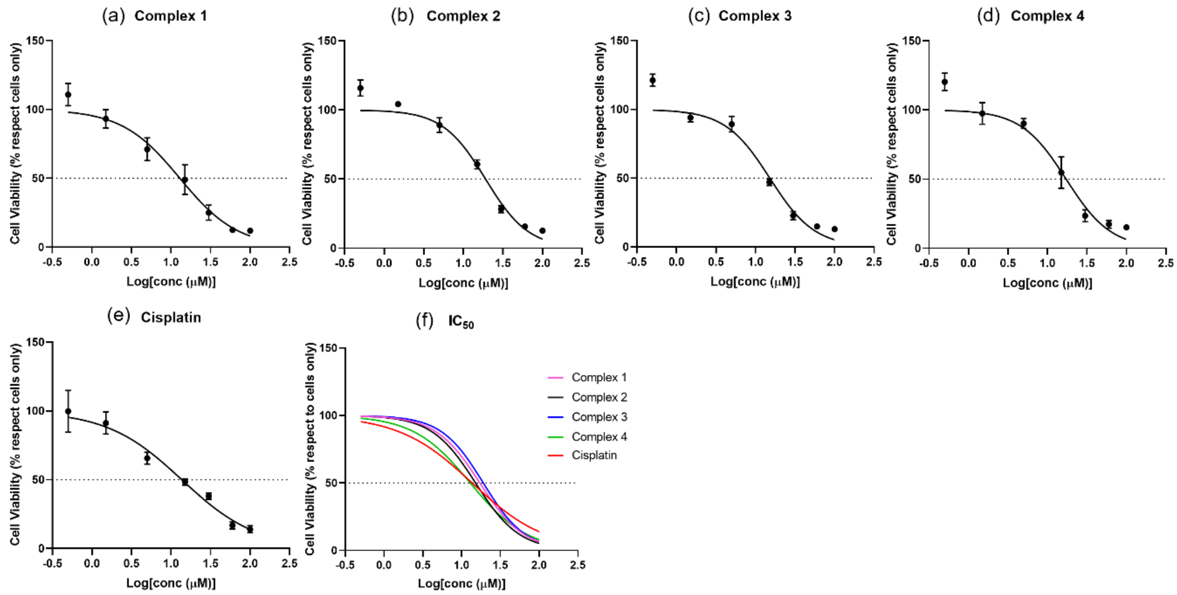

Figure S22: 2D *in vitro* screening of complexes 1-4 on SAOS-2 cell line by MTT assay and IC_{50} (μM) values. Dose-response curves used to generate IC_{50} (μM) for complexes 1 (a), 2 (b), 3 (c) and 4 (d), and for cisplatin (e) inhibitor activity on cell viability of SAOS-2 cell line. The Log[concentration] in μM and the normalized response (%) of survival fraction of cells are reported on X and Y asses, respectively. For each complex, the curve interpolation with 50% survival cells is highlighted in Y dotted line and correspond to its $LogIC_{50}$. A comparison of all dose-response curves is reported (f).

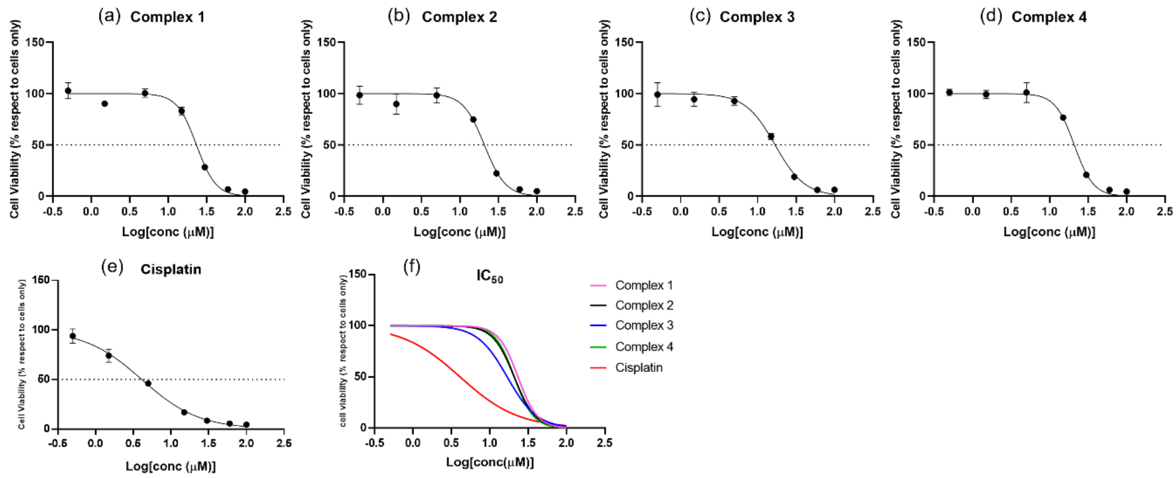

Figure S23: 2D *in vitro* screening of complexes 1-4 on hFOBs cell line by MTT assay and IC_{50} (μM) values. Dose-response curves used to generate IC_{50} (μM) for complexes 1 (a), 2 (b), 3 (c) and 4 (d), and for cisplatin (e) inhibitor activity on cell viability of hFOBs cell line. The Log[concentration] in μM and the normalized response (%) of survival fraction of cells are reported on X and Y asses, respectively. For each complex, the curve interpolation with 50% survival cells is highlighted in Y dotted line and correspond to its $LogIC_{50}$. A comparison of all dose-response curves is reported (f).

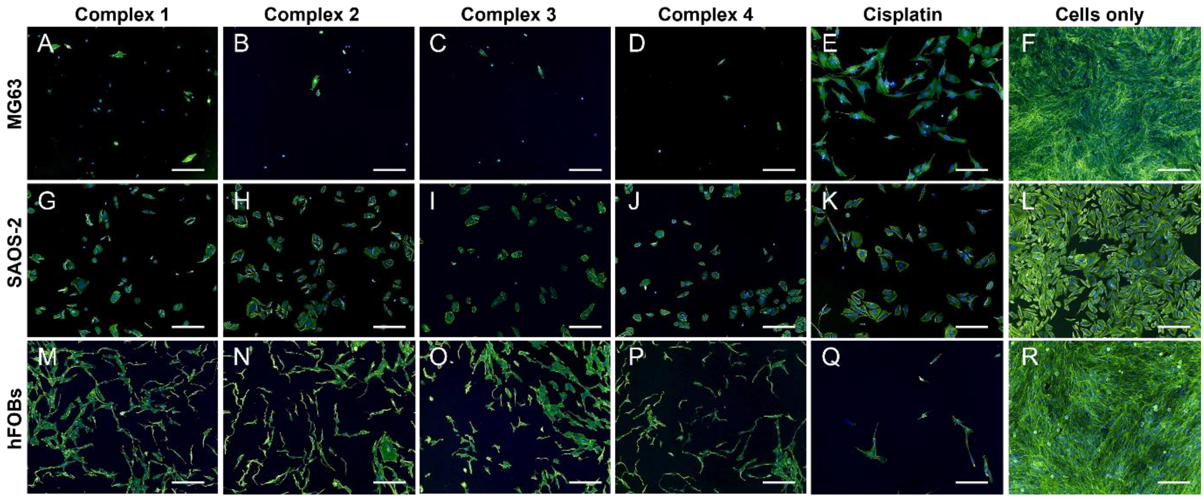

Figure S24: Actin and DAPI staining of 2D *in vitro* screening of complexes **1-4.** The cell morphology evaluation of MG63 (A – F), SAOS-2 (G – L) and hFOBs (M – R) cell lines cultured with complexes 1-4 and cisplatin are reported in the figure. For SAOS-2 and hFOBs 30 µM concentration was selected for the analysis, while 60 µM is reported for MG63 cells. F actin filaments in Phalloidin (Green) and cell nuclei in DAPI (Blue). Scale bars 200 µm.

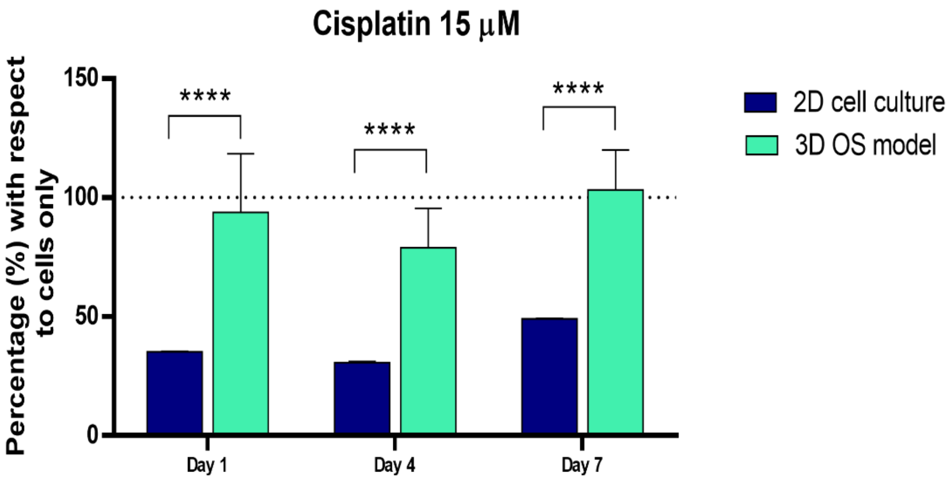

Figure S25: Anti-cancerous effect of cisplatin on 3D OS model *versus* 2D cell culture. Cell viability evaluation at day 1, 4 and 7 of culture by MTT assay. Data are reported in the graph as percentage (%) mean ± standard deviation. Significant differences of cisplatin cytotoxicity between different cell culture systems are reported in the graph at each time point ****p value ≤ 0.0001.

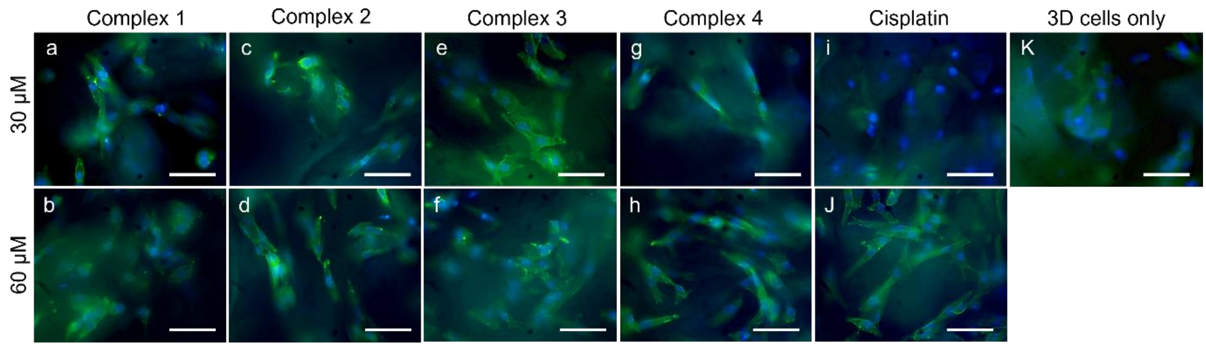

Figure S26: Actin and DAPI staining of *in vitro* 3D tumour-engineered models of osteosarcoma**.** Cell morphology evaluation of 3D tumour engineered models of MG63 cells after 72 hours in the presence of complex 1 (a;b), 2 (c;d), 3 (e;f), 4 (g;h) and cisplatin (i;j), and without any drug (k). F-Actin filaments in green (FITC) and cell nuclei in blue (DAPI). Scale bars 50 µm.