PAMAM-based mannose-glycodendrimers as multi-electron redox probes for improving lectin sensing

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Sulfuric Acid-Phenol assays: A sulfuric acid-phenol assay[1] was used to determine the number of mannose units in compounds **12** and **13**. Briefly, concentrated sulfuric acid (600 µL) and a solution of 5 % phenol in water (120 µL) were added to an aqueous solution of D-mannose or compounds **12-13** (200 µL). The resulting solutions were incubated at 90 ºC for 5 min and then cooled down to room temperature in a water bath for another 5 min. Finally an UV-visible spectrum was recorded and the absorbance was measured at 487 nm. A calibration curve was done using D-mannose as reference (Figure S1).

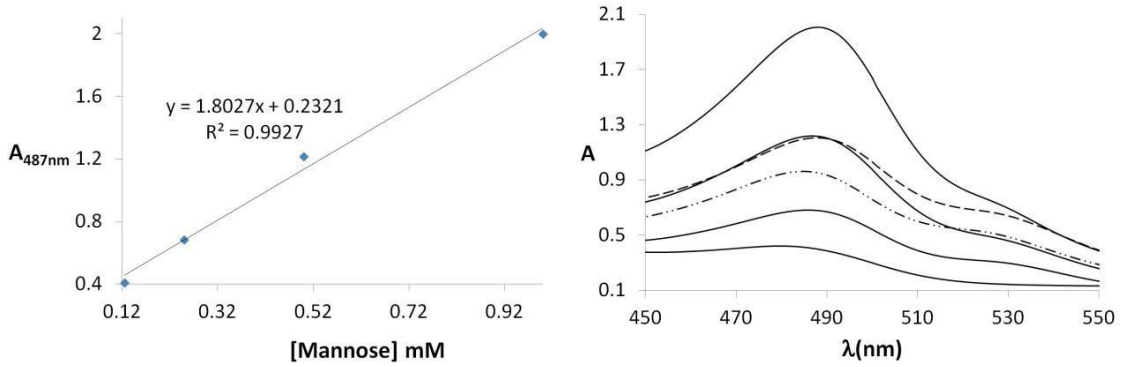

Figure S1. Calibration curve for D-mannose (left), and UV-visible spectra of mannose (—, four samples between 0.12 and 1 mM), compound **12** (—··—) and compound **13** (— —) after being treated with sulfuric acid-phenol method.

Dendrimer (mg)	Equation[a]	V[b] (mL)	A (487 nm)	[Mannose units] (mM)	n
12 (6.8 ± 0.1)	M_{Man}/M_t= n·218.20/(3568.38 + n·218.20)	25	0.9636	0.443	7.90 ± 0.17
14 (9.3 ± 0.1)	M_{Man}/M_t= n·218.20/(7529.21 + n·218.20)	25	1.2045	0.539	15.97 ± 0.24

[a]M_{Man}= Molecular weight of the mannose moiety (highlighted in red in Figure S2 for **12** and S3 for **13**); M_t=Molecular weight of ferrocenylated dendrimer core (in black in Figure S2 for **12** and S3 for **13**); n = number of mannose units, [b]MilliQ water.

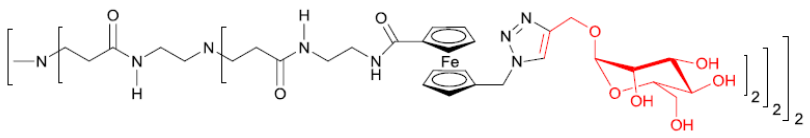

Figure S2. Chemical structure of compound **12**.

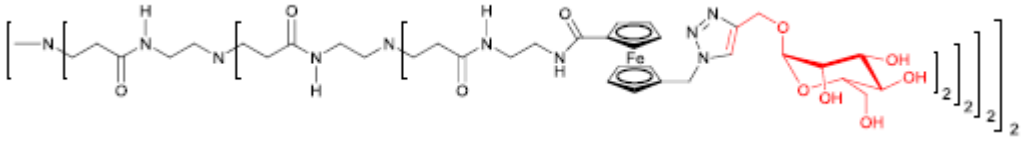

Figure S3. Chemical structure of compound **13**.

^{1}H-NMR and ^{13}C-NMR spectra for compounds 2, 6-8 and 10-12, and mass spectra for compounds 6-8 and 10:

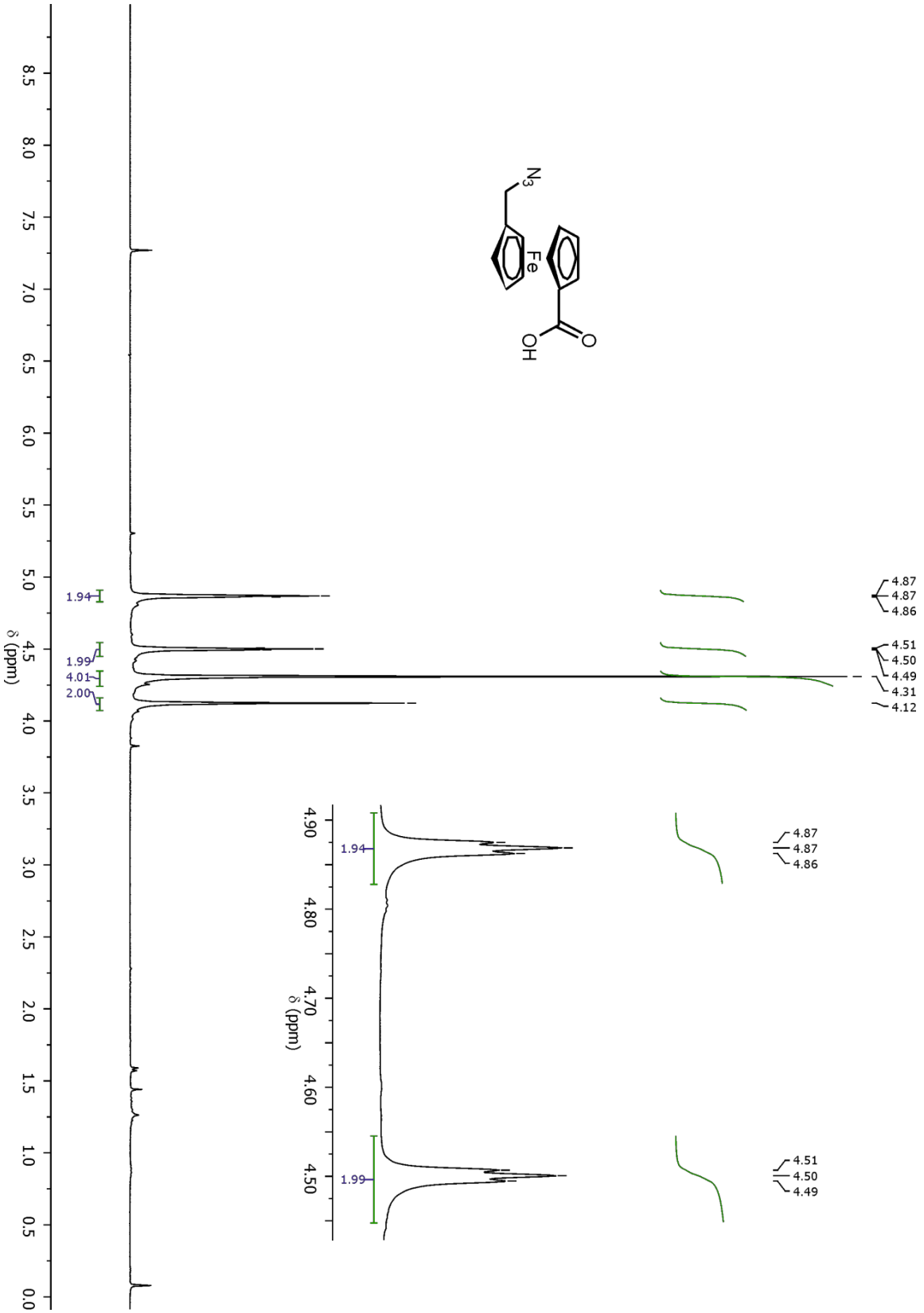

Figure S4. ^{1}H-NMR spectrum (300 MHZ, $CDCl_3$, 25 ºC) for compound **2**.

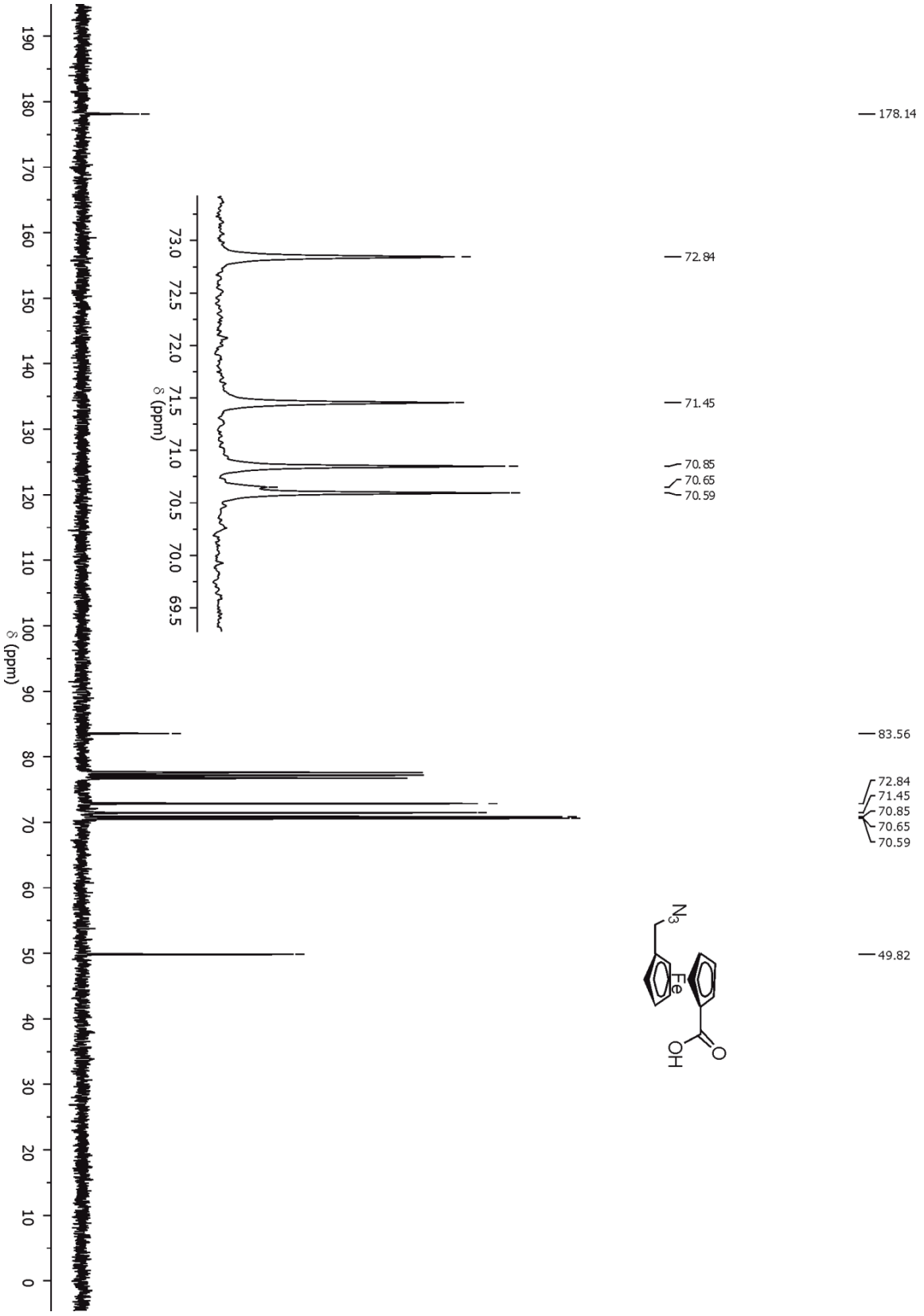

Figure S5. ^{13}C-NMR spectrum (75 MHZ, $CDCl_3$, 25 ºC) for compound **2**.

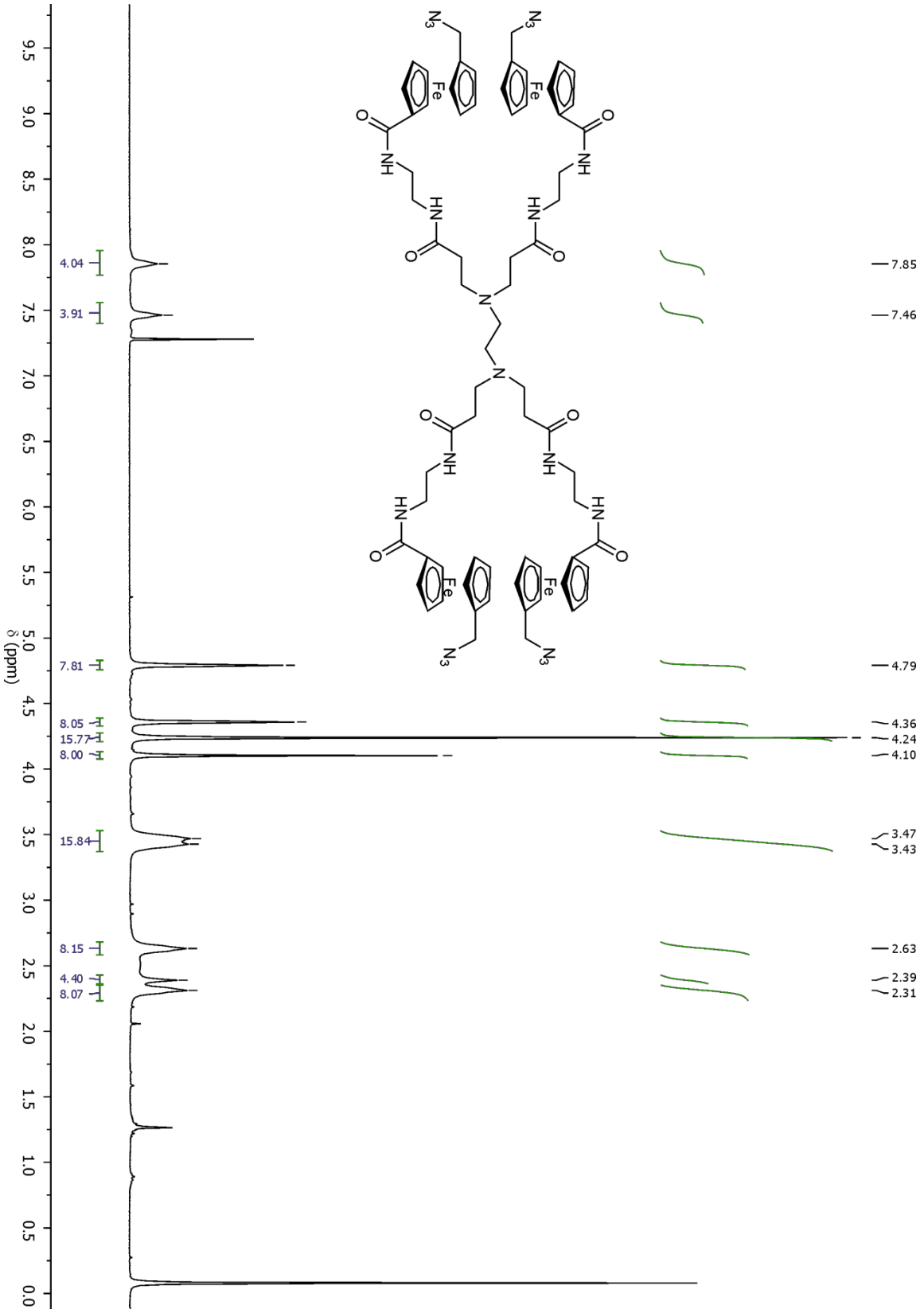

Figure S6. ^{1}H-NMR spectrum (300 MHZ, $CDCl_3$, 25 ºC) for compound **6**.

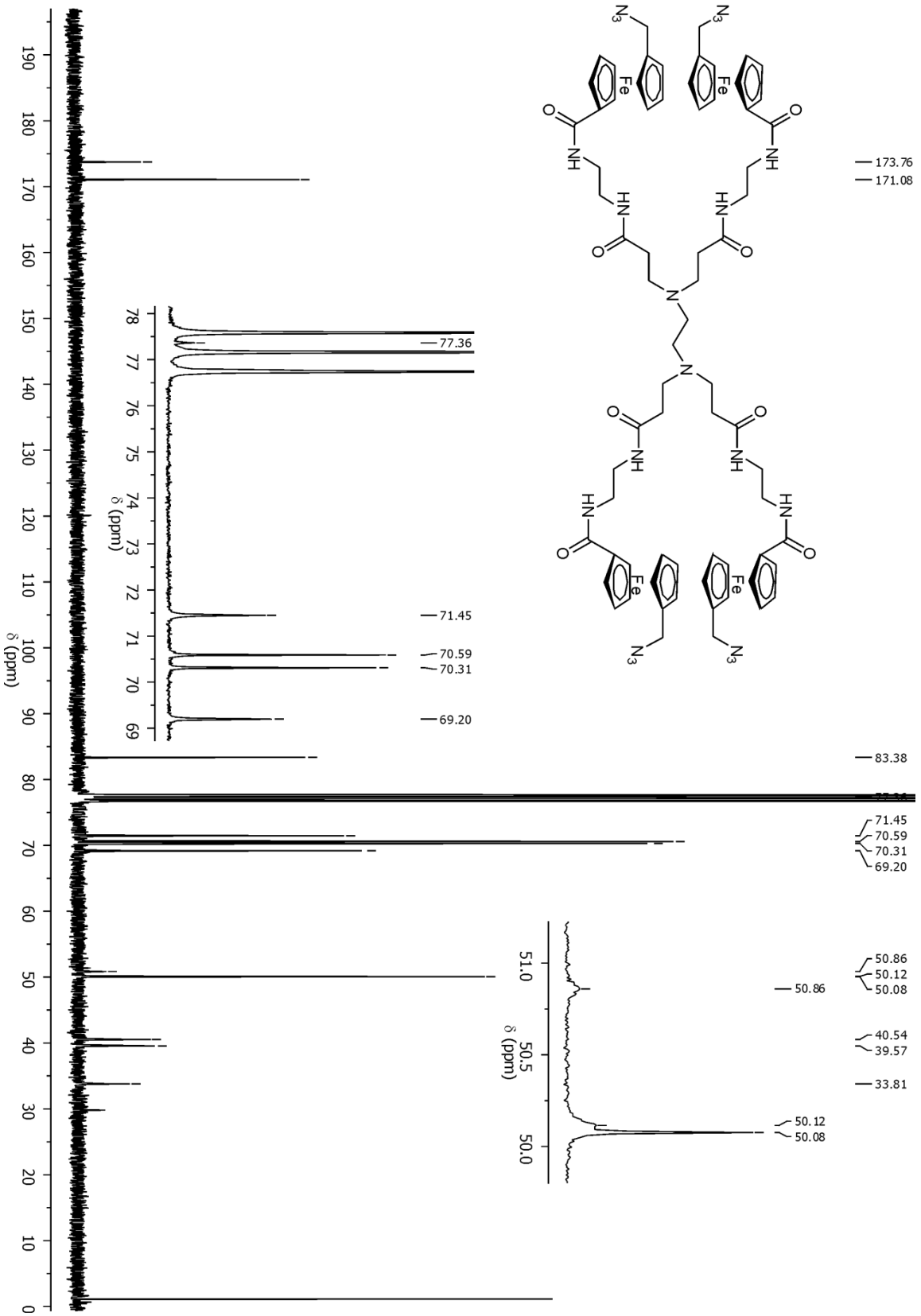

Figure S7. ^{13}C-NMR spectrum (75 MHZ, $CDCl_3$, 25 ºC) for compound **6**.

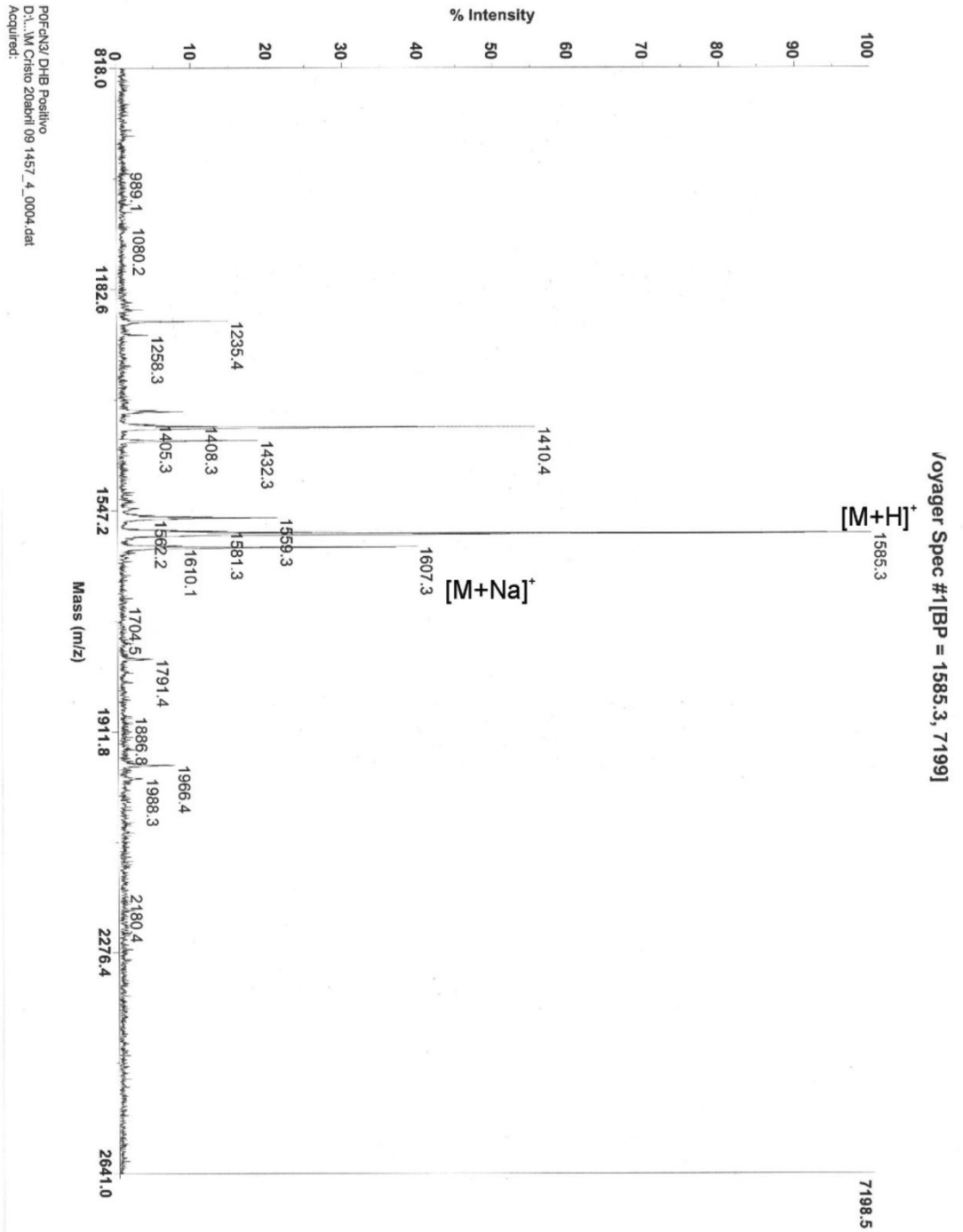

Figure S8. MALDI-TOF spectrum for compound **6**.

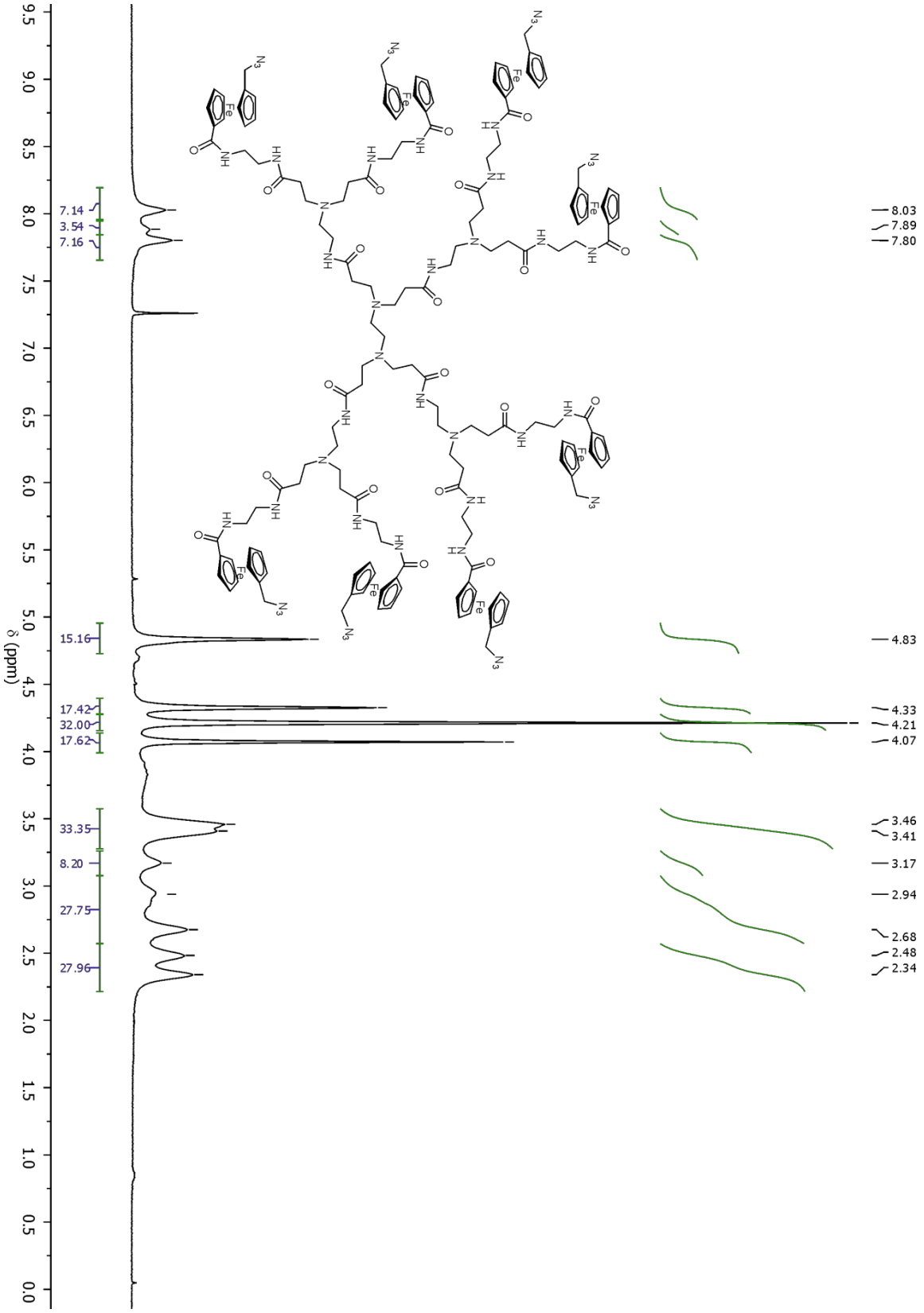

Figure S9. ^{1}H-NMR spectrum (300 MHZ, $CDCl_3$, 25 ºC) for compound **7**.

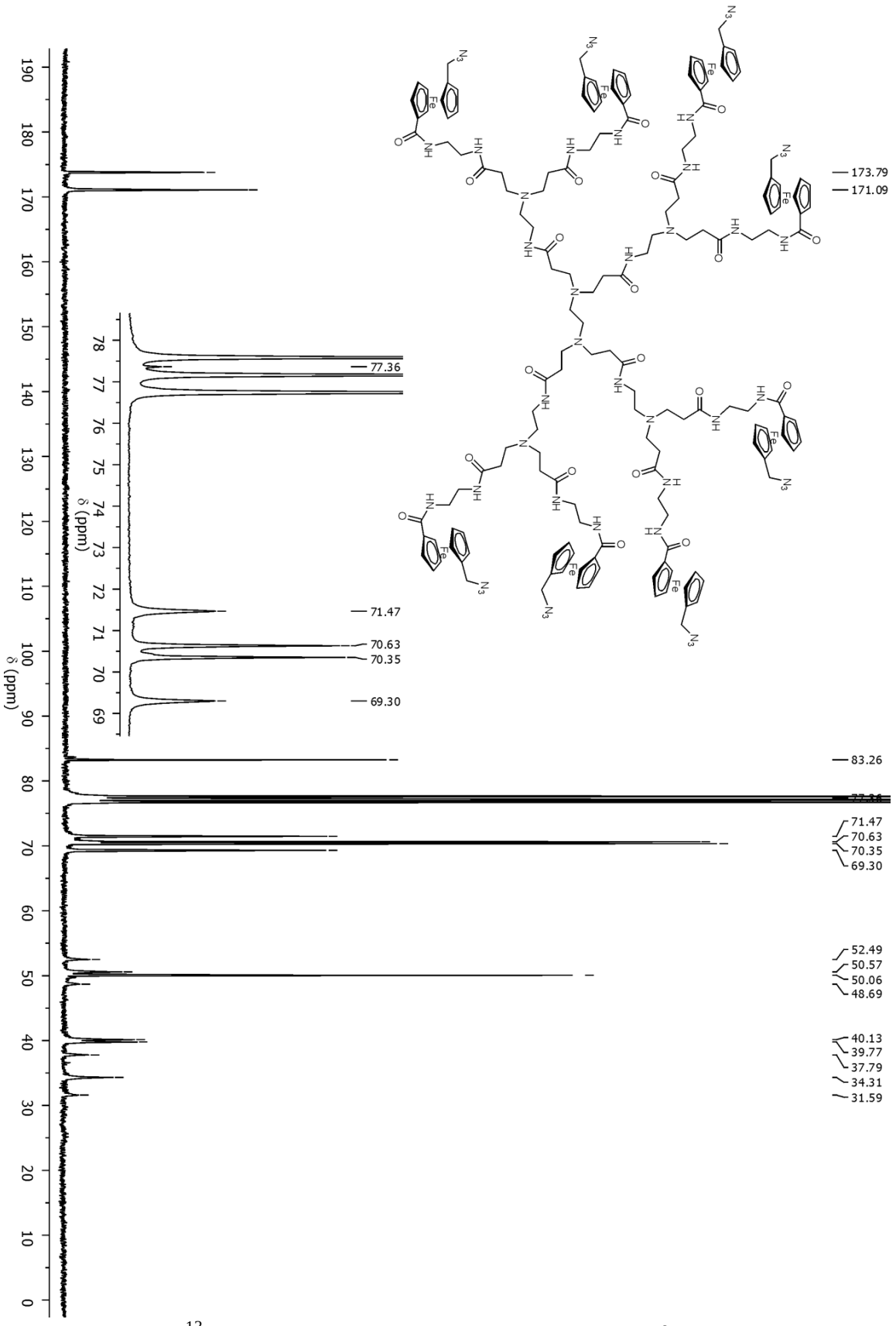

Figure S10. ^{13}C-NMR spectrum (75 MHZ, $CDCl_3$, 25 ºC) for compound **7**

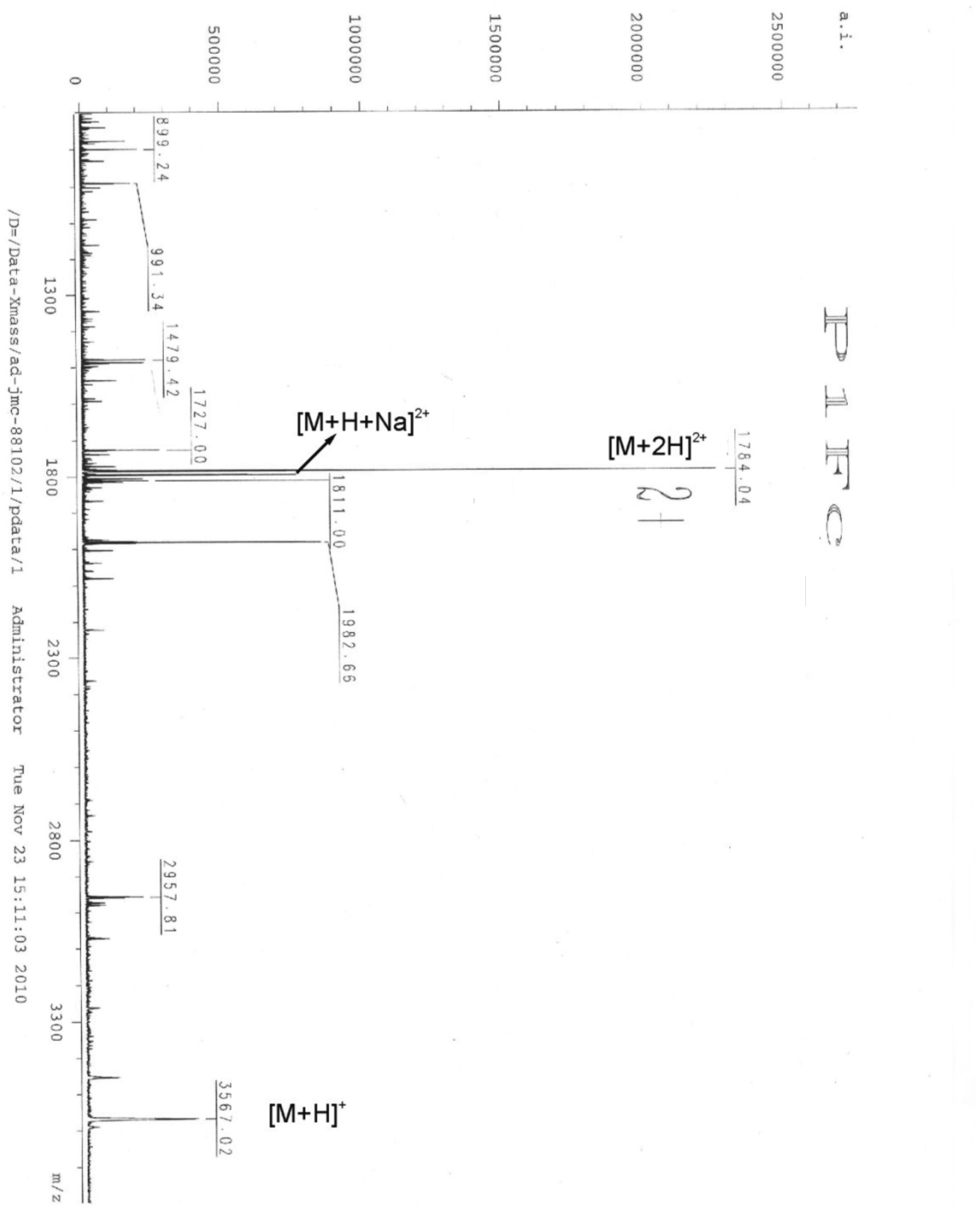

Figure S11. ESI-TOF mass spectrum for compound **7**.

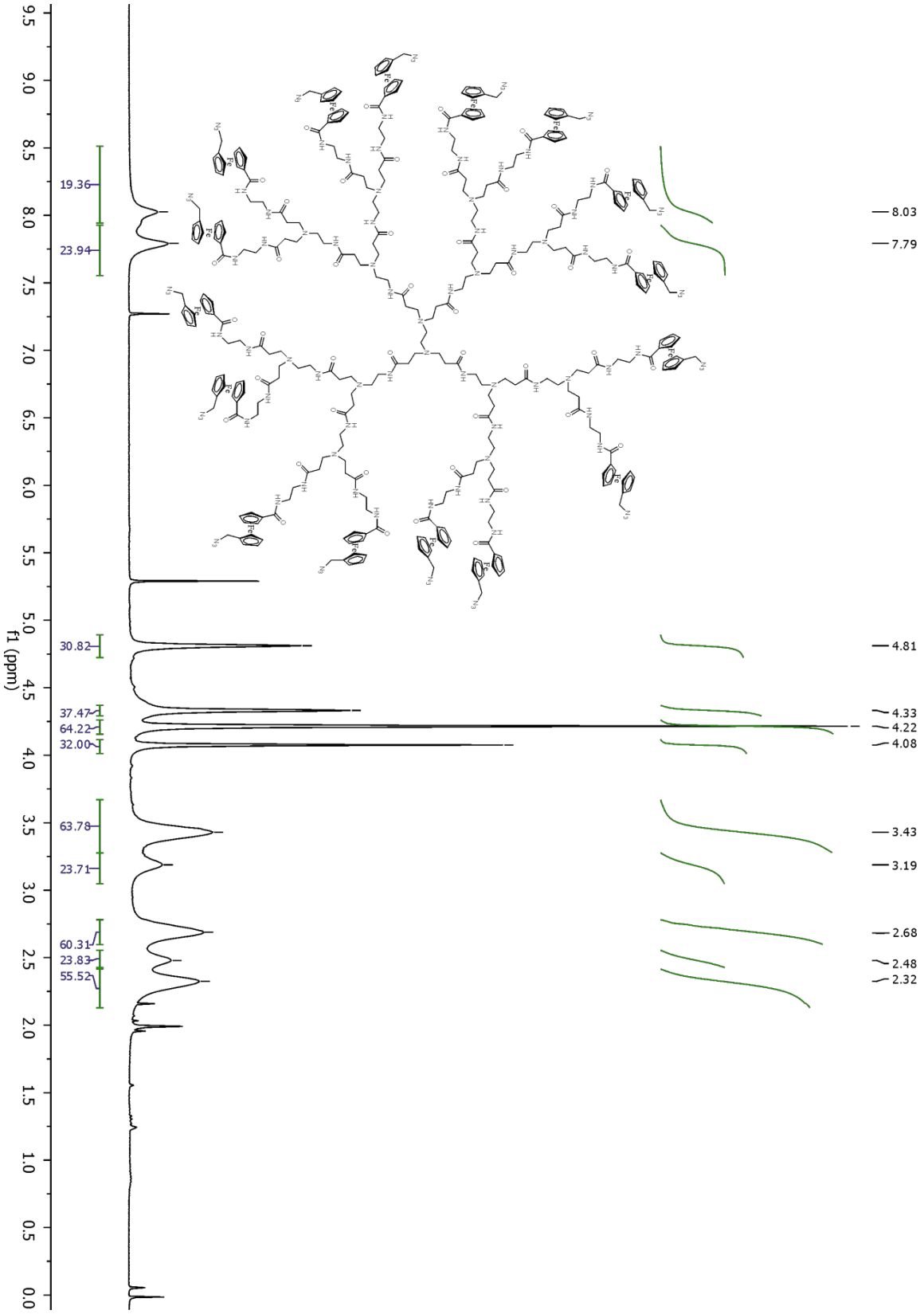

Figure S12. ^{1}H-NMR spectrum (300 MHZ, $CDCl_3$, 25 ºC) for compound **8**.

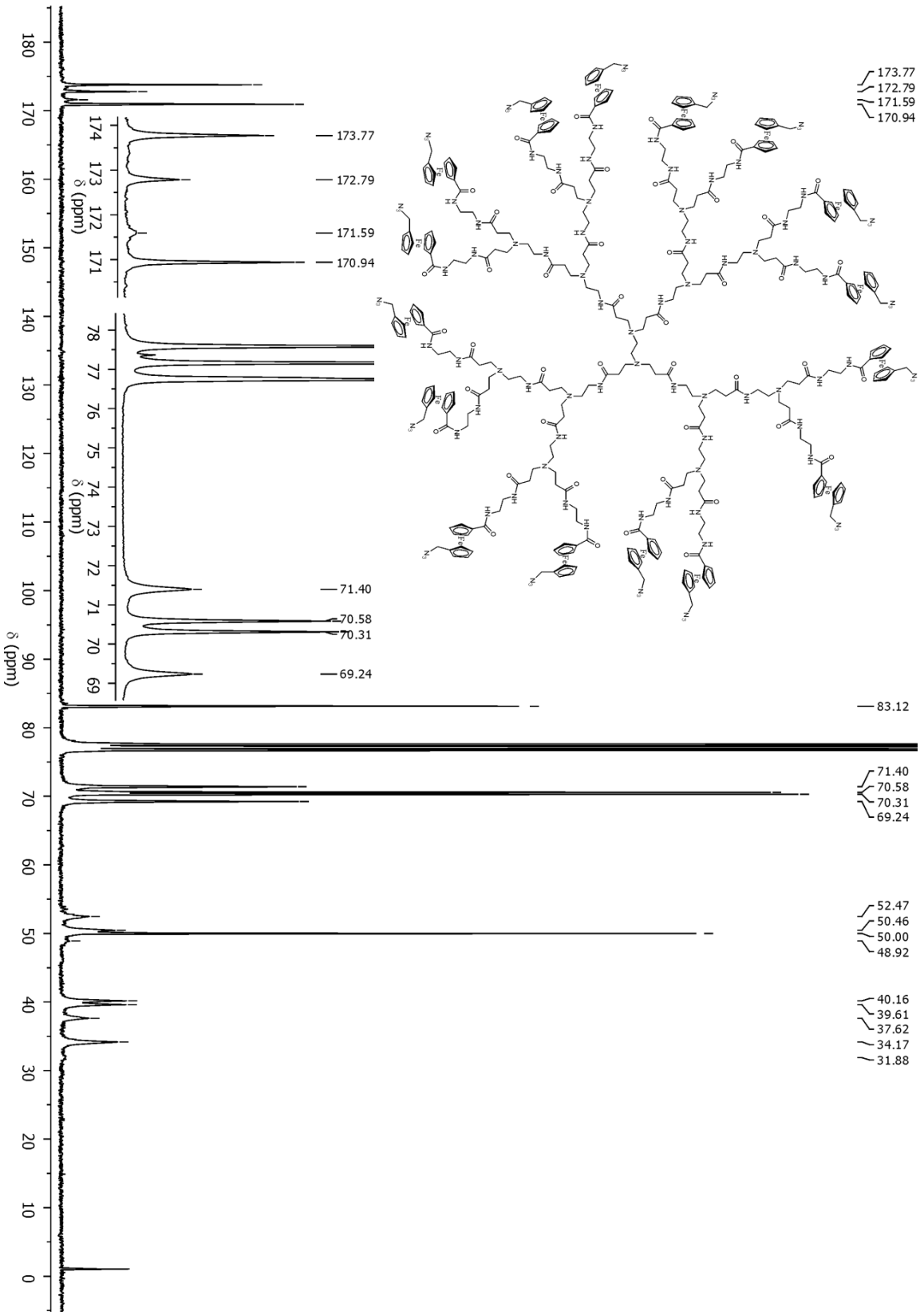

Figure S13. ^{13}C-NMR spectrum (75 MHZ, $CDCl_3$, 25 ºC) for compound **8**.

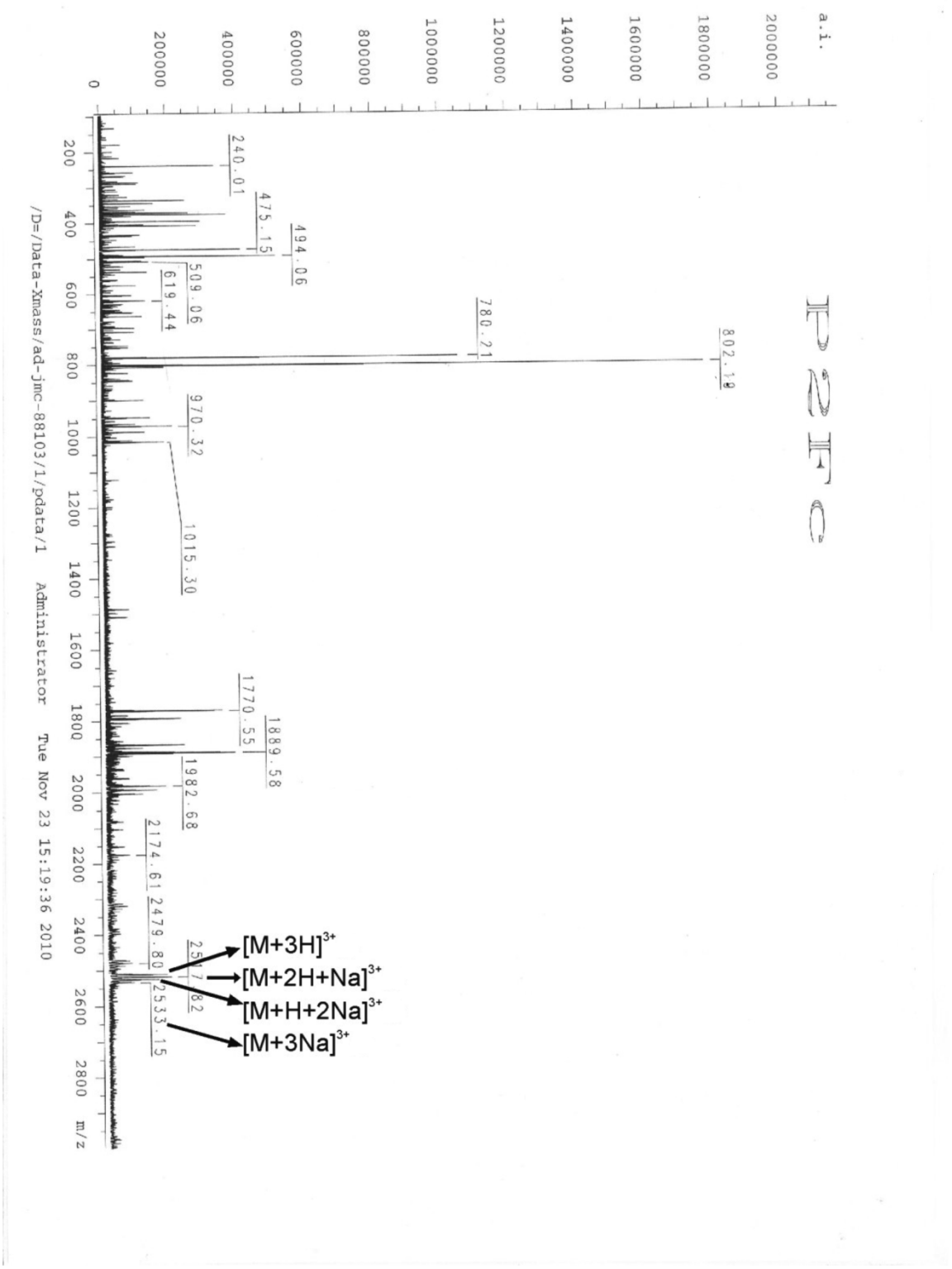

Figure S14. ESI-TOF mass spectrum for compound **8**.

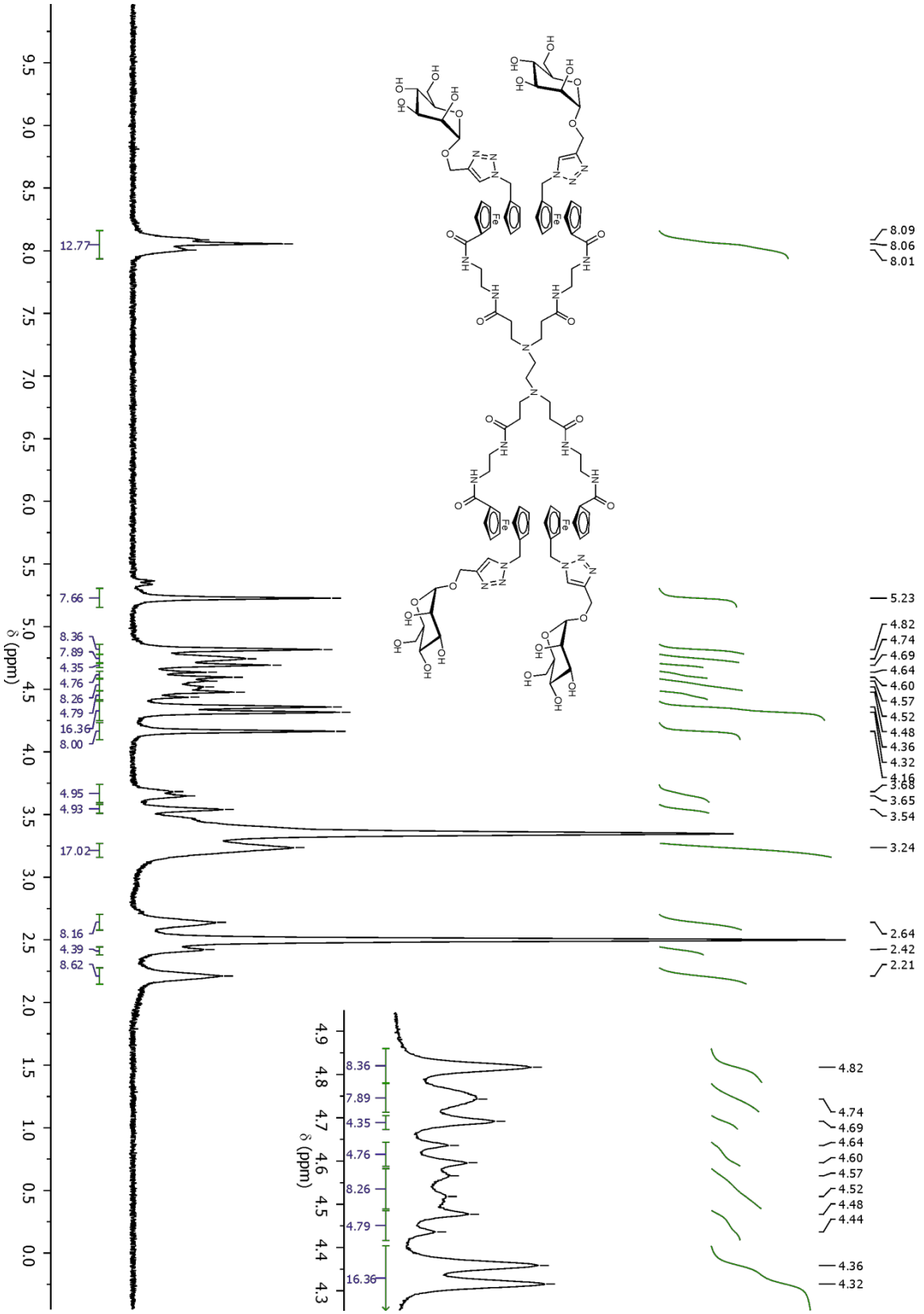

Figure S15. ^{1}H-NMR spectrum (300 MHZ, DMSO-d_6, 25 ºC) for compound **10**.

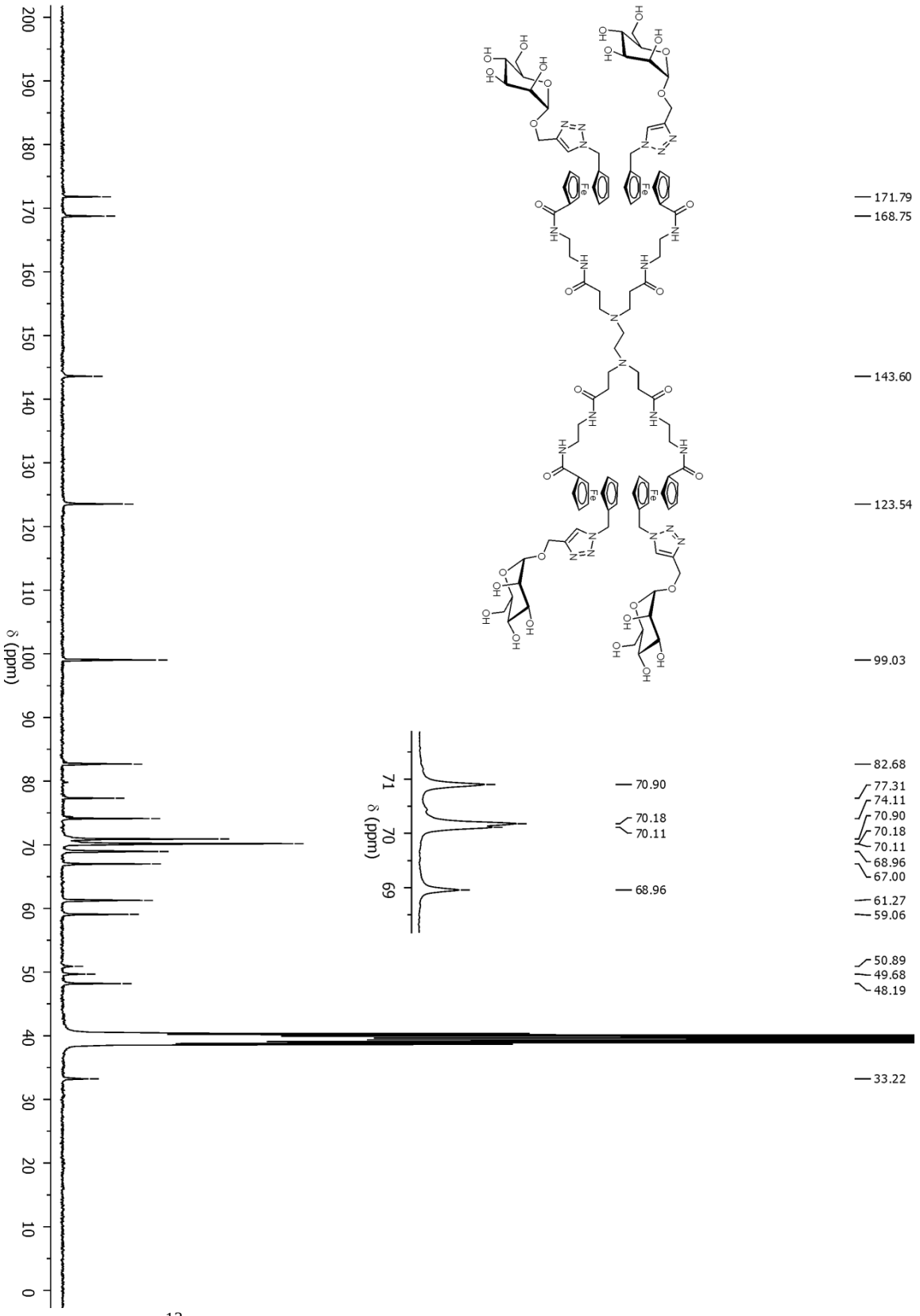

Figure S16. ^{13}C-NMR spectrum (75 MHZ, DMSO-d_6, 25 ºC) for compound **10**.

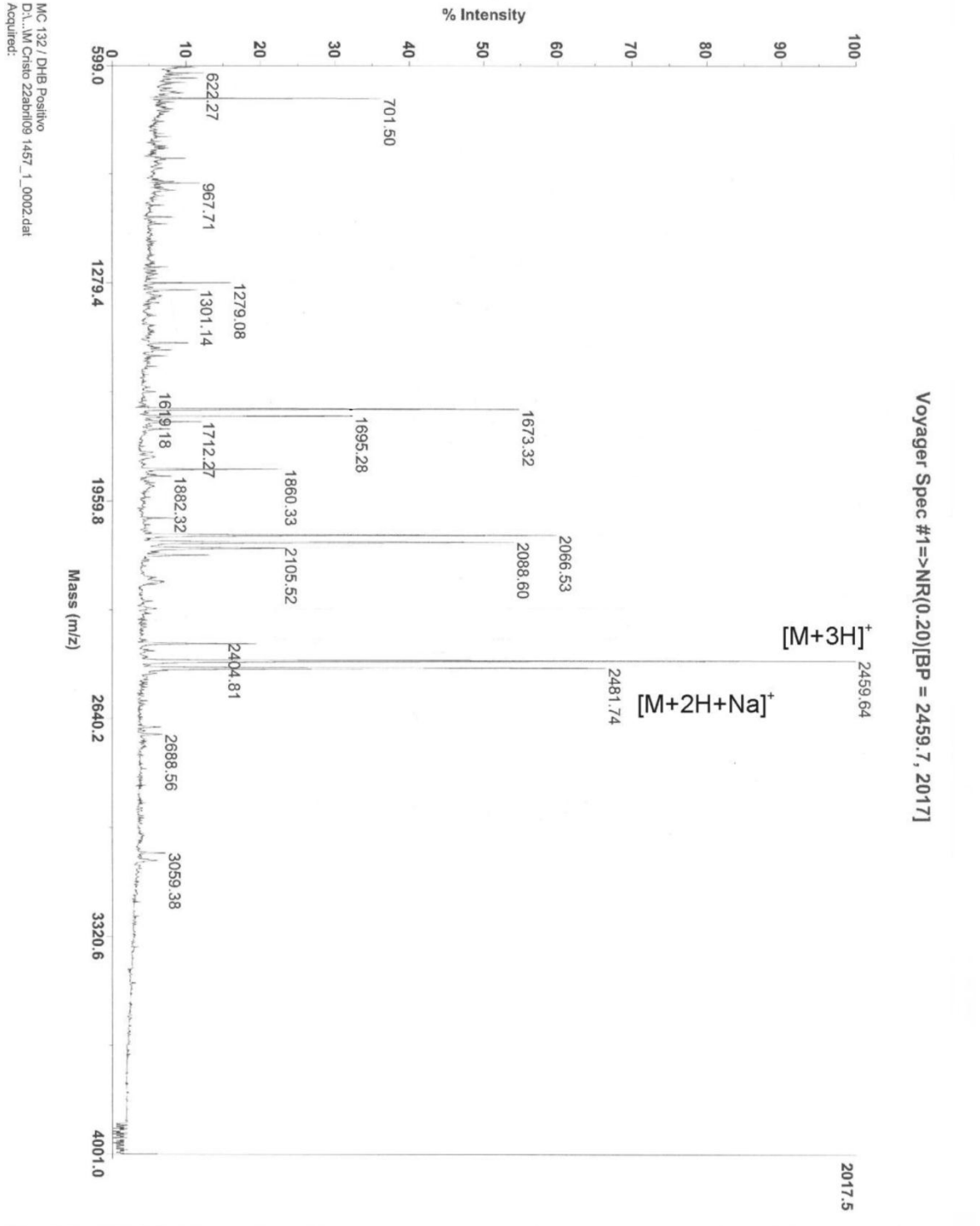

Figure S17. MALDI-TOF spectrum for compound **10**.

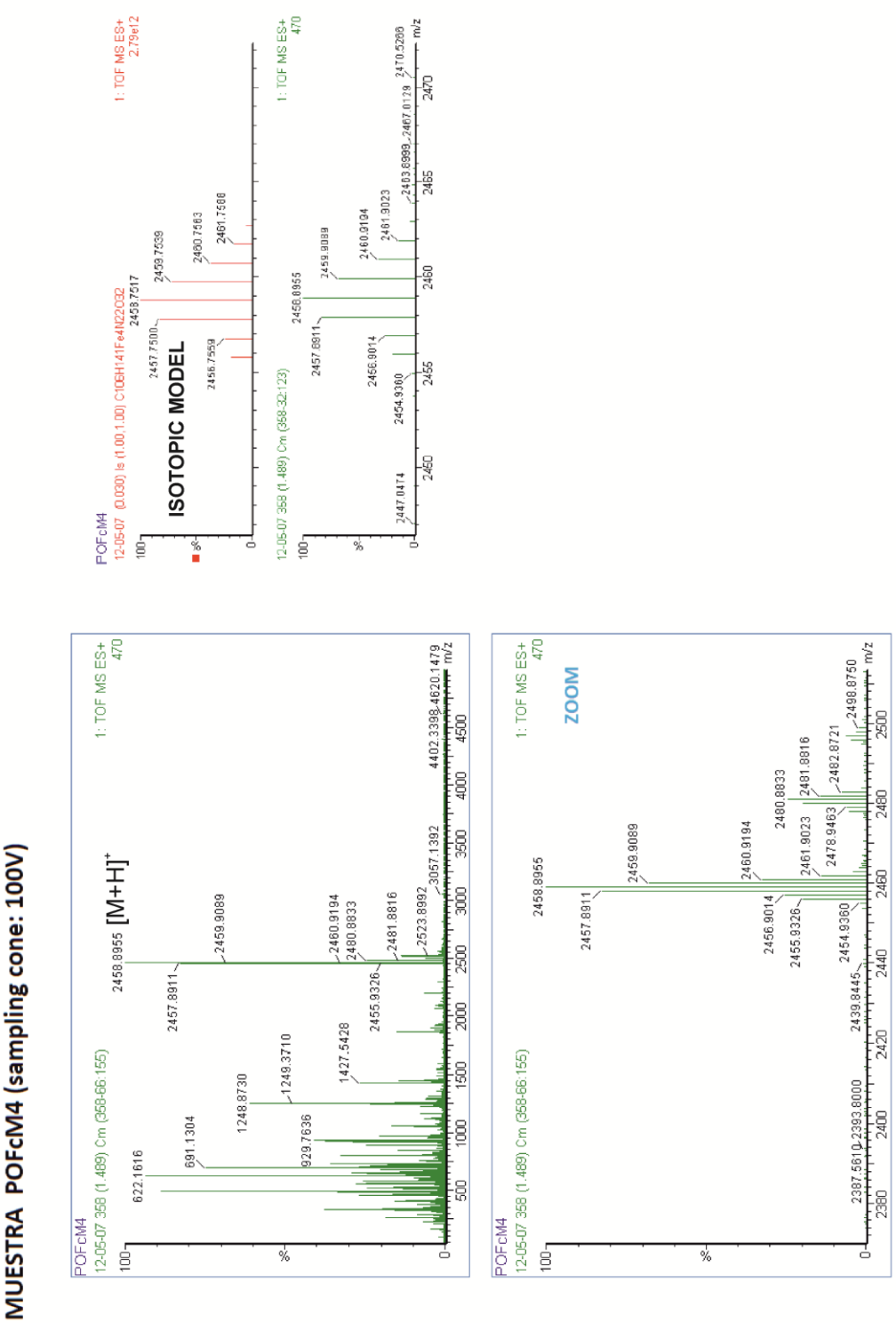

Figure S18. ESI-TOF mass spectrum for compound **10**.

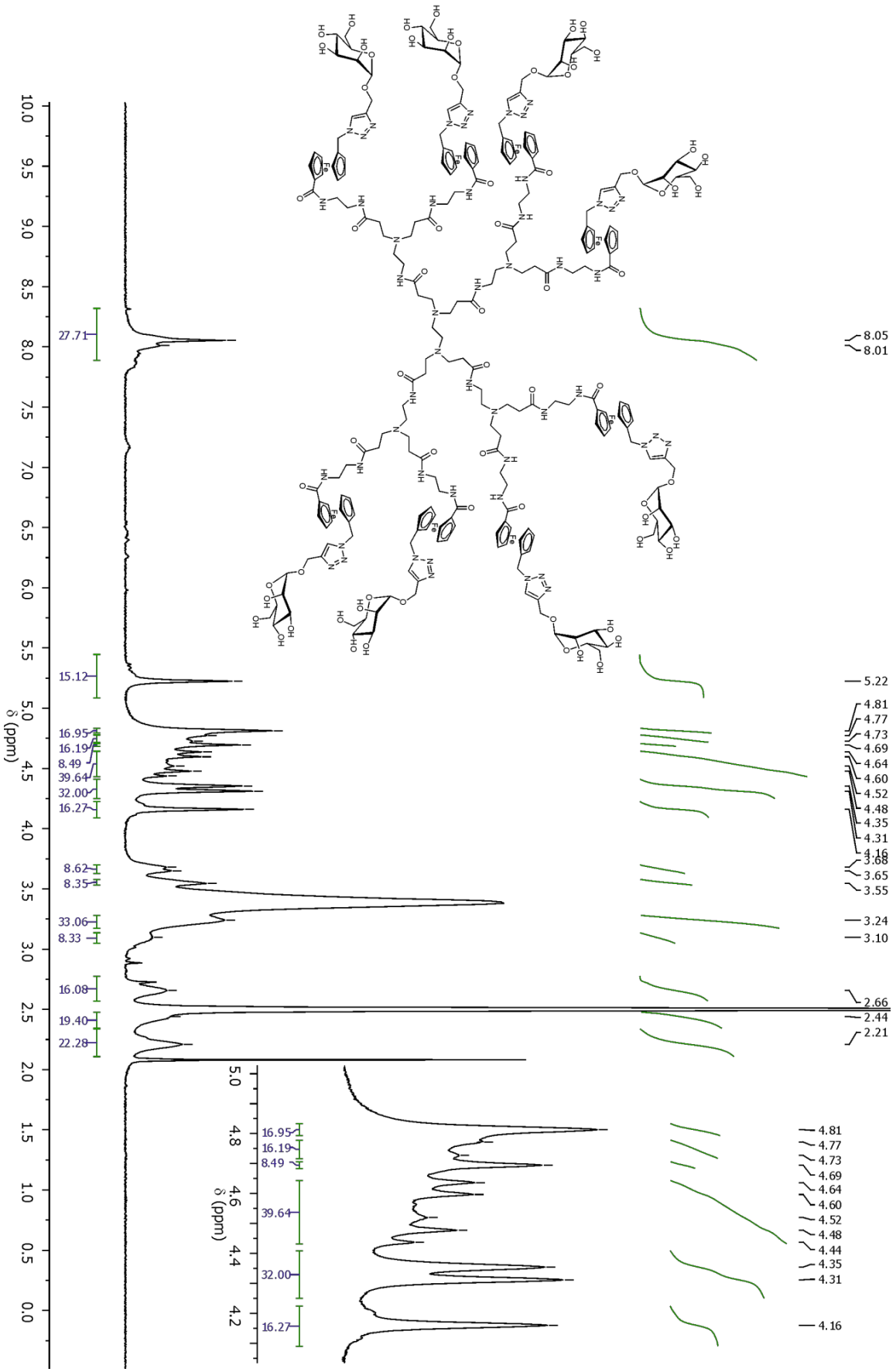

Figure S19. ^{1}H-NMR spectrum (300 MHZ, DMSO-d_6, 25 ºC) for compound **11**.

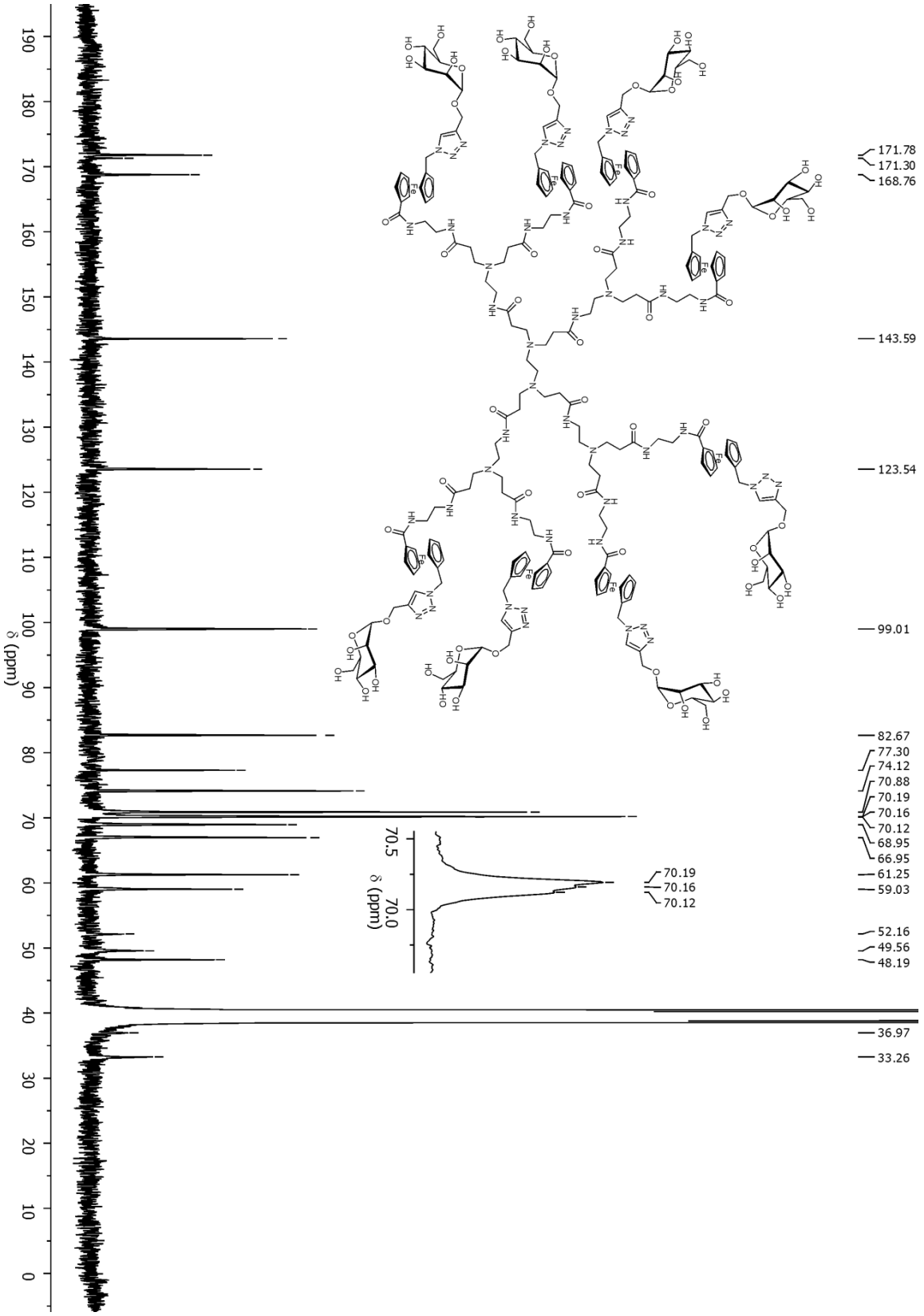

Figure S20. ^{13}C-NMR spectrum (75 MHZ, DMSO-d_6, 25 ºC) for compound **11**.

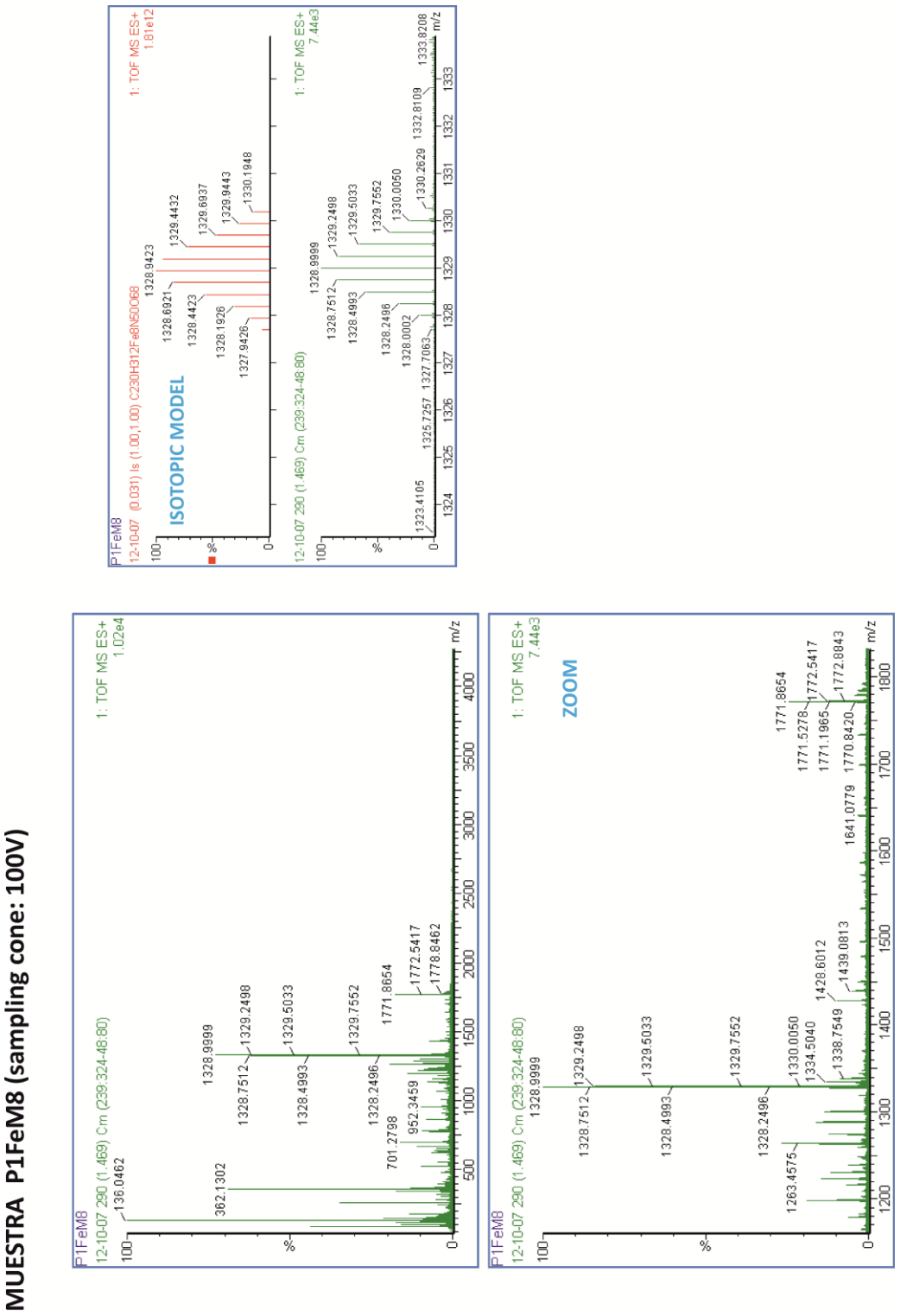

Figure S21. ESI-TOF mass spectrum for compound **12**.

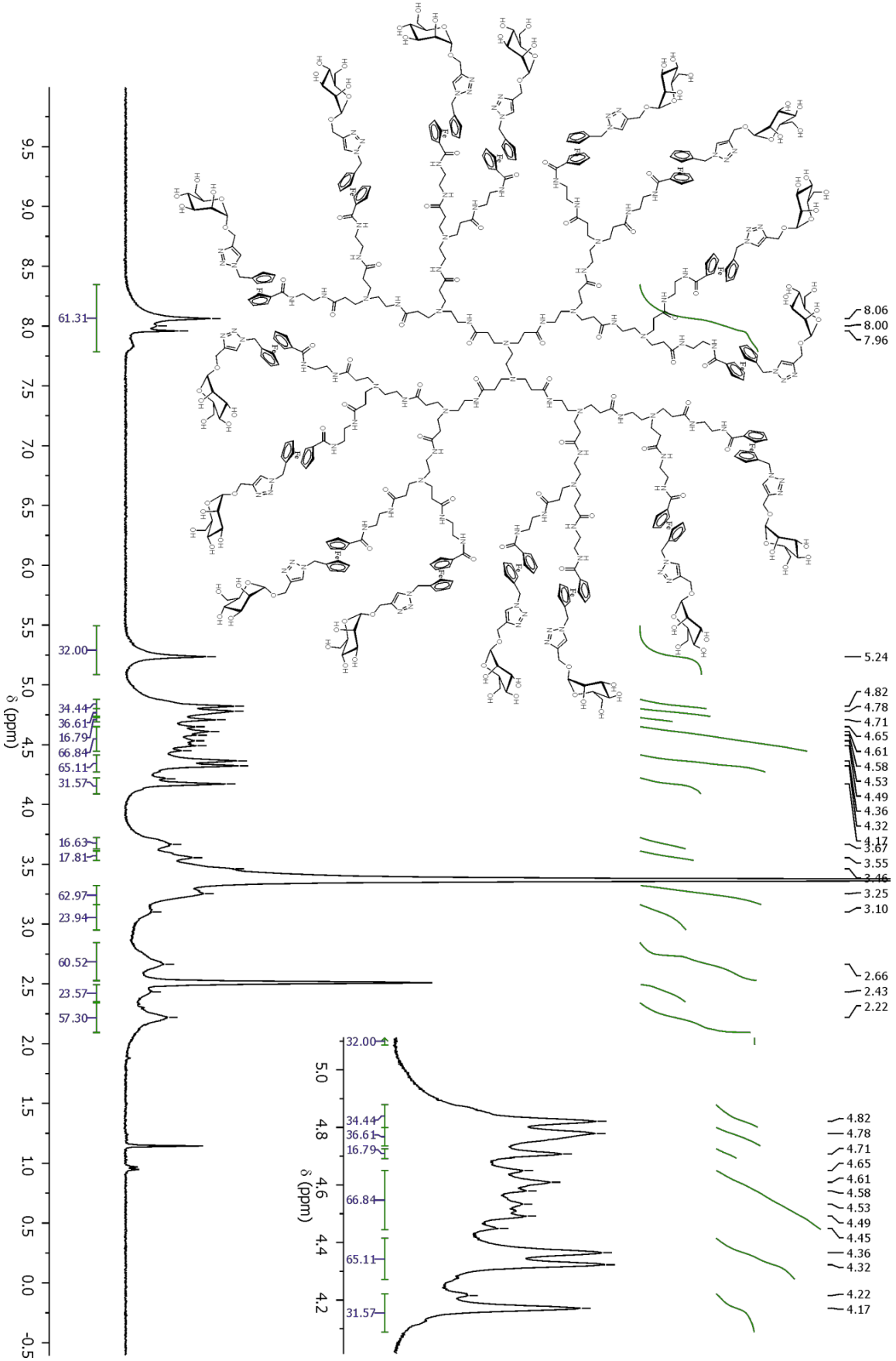

Figure S22. ^{1}H-NMR spectrum (300 MHZ, DMSO-d_6, 25 ºC) for compound **12**.

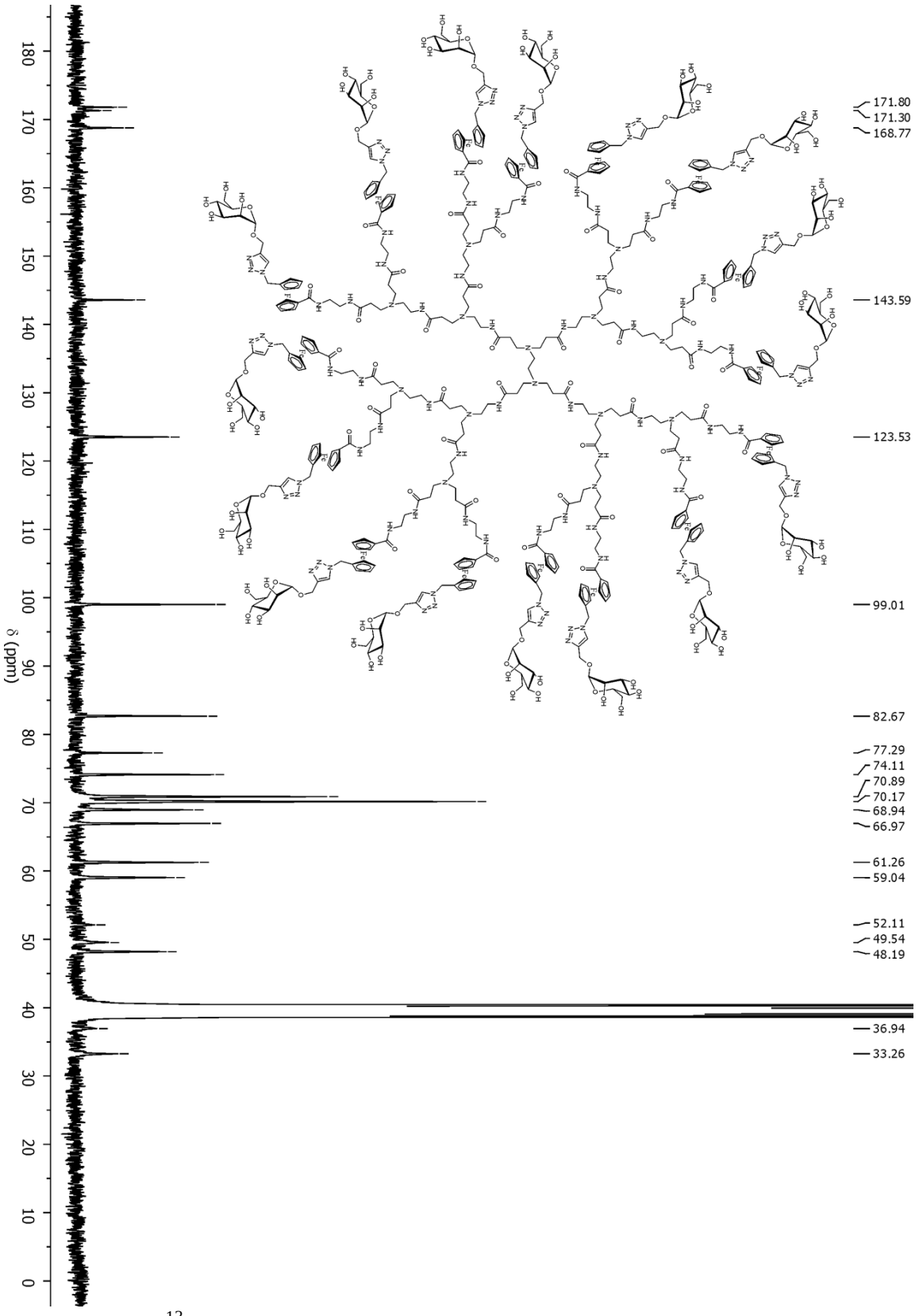

Figure S23. ^{13}C-NMR spectrum (75 MHZ, DMSO-d_6, 25 ºC) for compound **12**.

IR spectra for compounds 6-8:

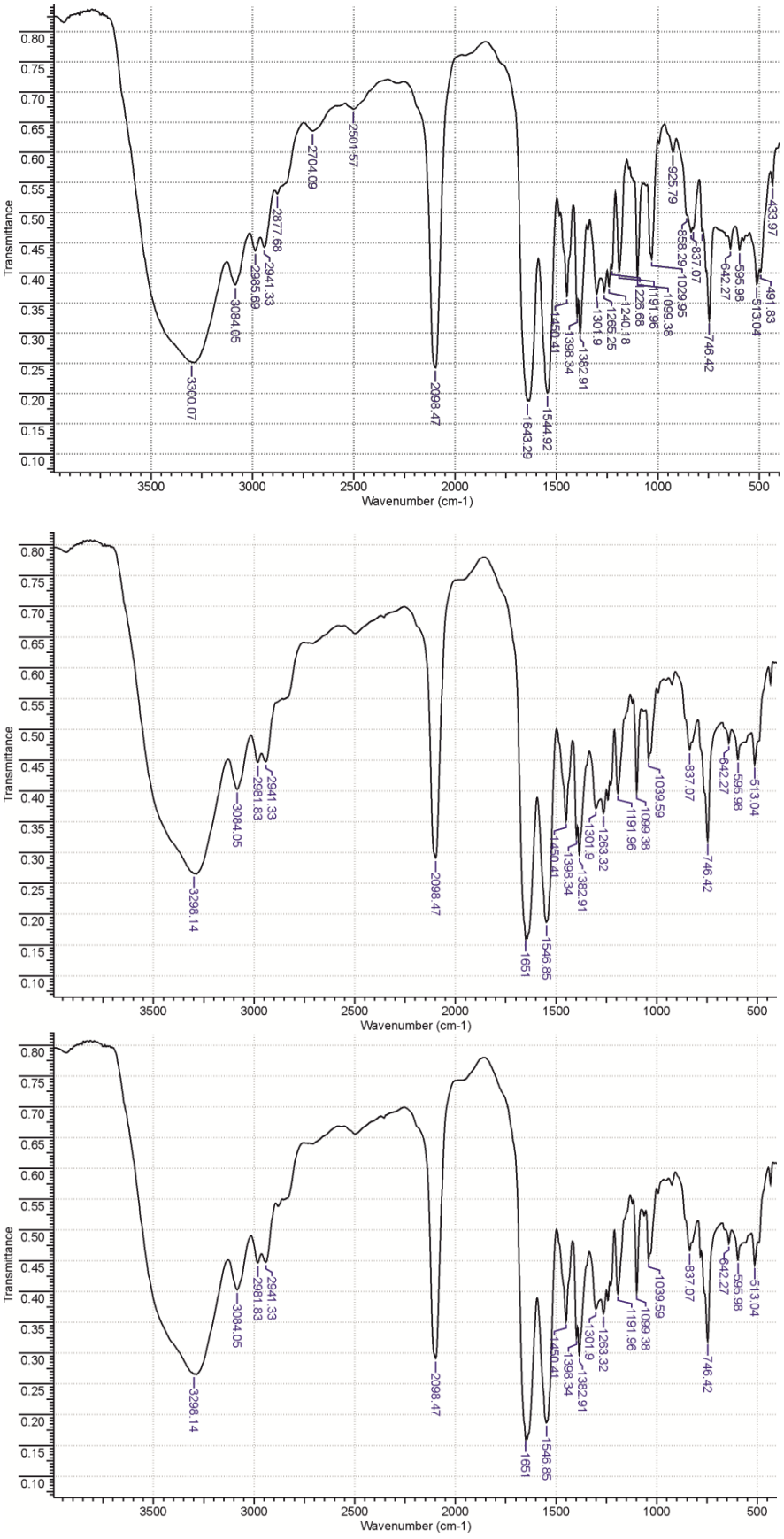

Figure S24. IR spectra of compound **6-8** (from top to bottom) in KBr.

IR spectra for compounds 10-12:

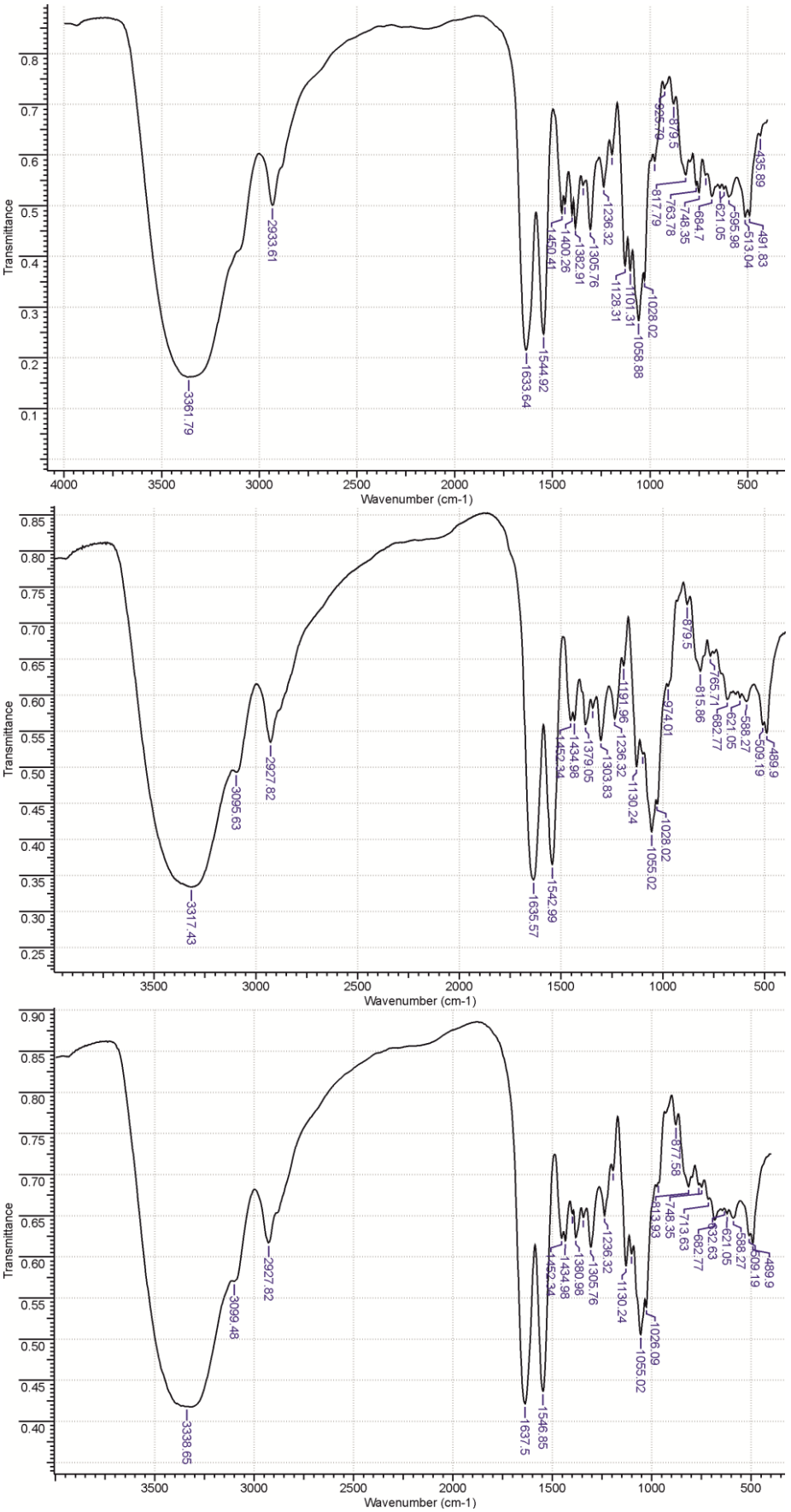

Figure S25. IR spectra of compound **10-12** (from top to bottom) in KBr.

DPV titrations for compounds 11-14:

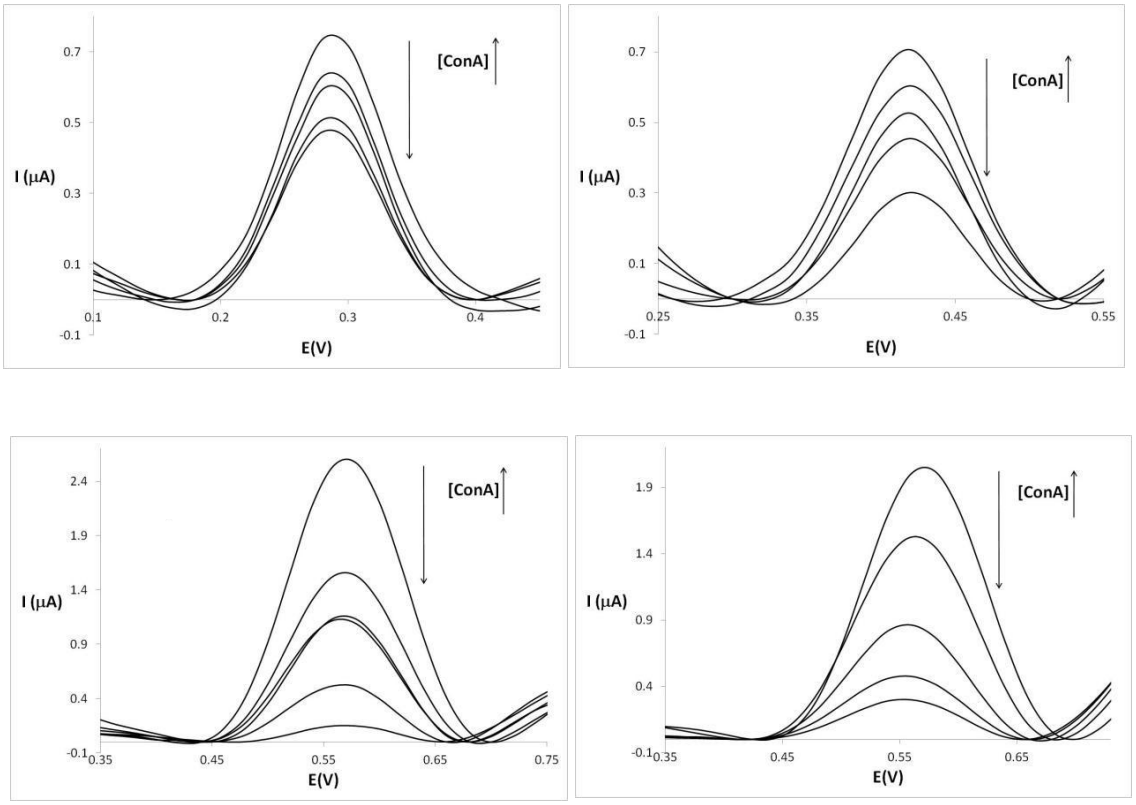

Figure S26. Differential pulse voltammograms for compounds **13** (top left), **14** (top right), **11** (bottom left) and **12** (bottom right) in the presence of increasing amounts of Concanavalin A (0-50 μM) in 10 mM TRIS buffer (pH 7.2) with 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$ and 200 mM NaCl for **11**, **13** and **14**, and with 20 mM NaCl for **12**.

ITC titrations for compounds 10 and 12:

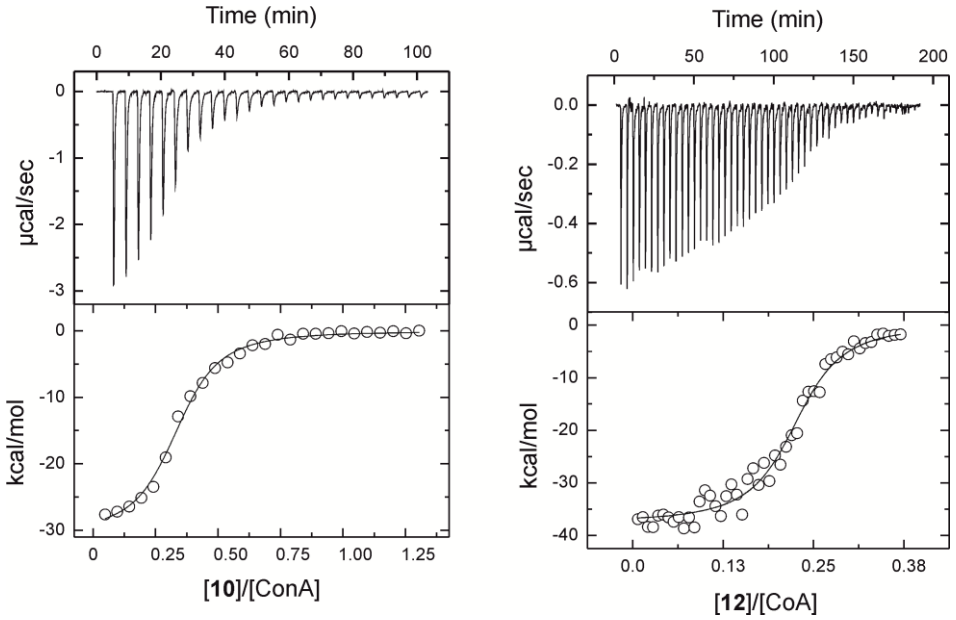

Figure S27. Titrations of Concanavalin A with compounds **10** (left), and **12** (right) in 10 mM TRIS buffer (pH 7.2) with 20 mM NaCl, 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$ at 25 ºC. The top panel shows the raw calorimetric data, denoting the amount of generated heat (negative exothermic peaks) following each injection of the solution of conjugates (600 µM for **10** and 100 µM for **12**) into the cell containing a solution of Concanavalin A (45 µM for **10** and 52 µM for **12**). The area under each peak represents the amount of heat released upon binding of the conjugates to the lectin. The smooth solid lines represent the best fit of the experimental data to a model of *n* equal and independent sites.

Differential pulse voltammograms and ITC thermograms for BSA control experiments:

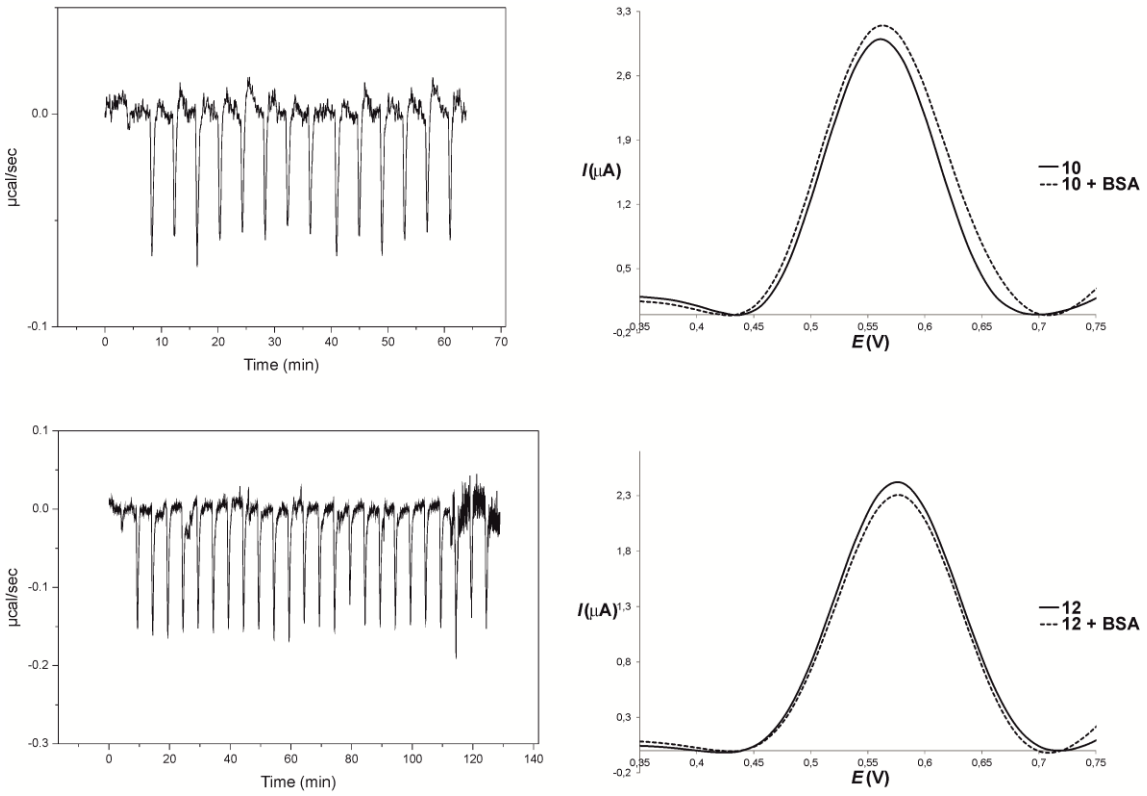

Figure S28. Left: Thermograms for the titration of BSA (47 µM) with compounds **10** (top, 600 µM), and **12** (bottom, 100 µM) in 10 mM TRIS buffer (pH 7.2) with 20 mM NaCl at 25 ºC. Right: Differential pulse voltammograms for compounds **10** (top) and **13** (bottom) in the absence (normal line) and the presence (dashed line) of BSA (40 µM) in 10 mM TRIS buffer (pH 7.2) with 200 mM NaCl for **10** and 20 mM NaCl for **13**.

References:

[1] T. Masuko, A. Minami, N. Iwasaki, T. Majima, S.-I. Nishimura, Y.-C. Lee, *Anal. Biochem.* **2005**, *339*, 69-72.