## Supplementary materials

# Biological effects in cancer cells of mono and bidentate conjugation of cisplatin on PAMAM dendrimers: a comparative study

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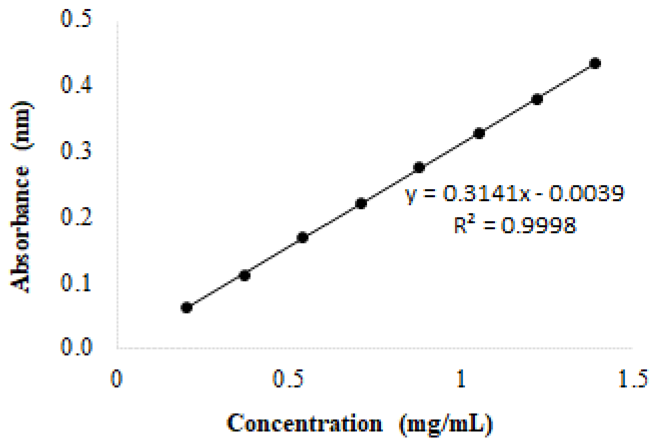



**Figure S1:** Standard curve of Hg using several dilutions: 0.2; 0.37; 0.54; 0.71; 0.88; 1.05; 1.22 and 1.39 mg/mL. The absorbance was measured at 550 nm.

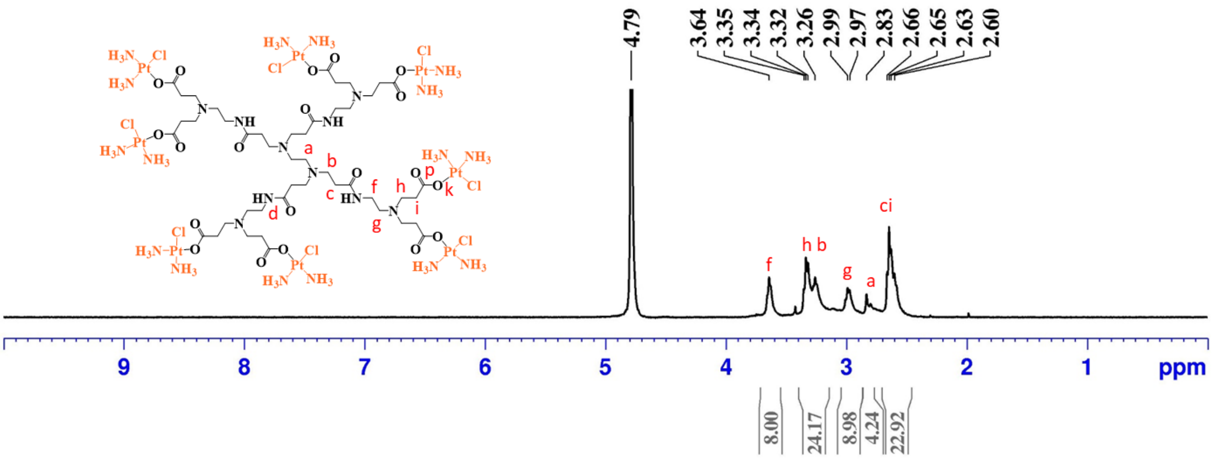



**Figure S2:** [1]H-NMR spectrum of monodentate G0.5(COOPt$(NH_3)_2$Cl$)_8$ in $D_2O$.

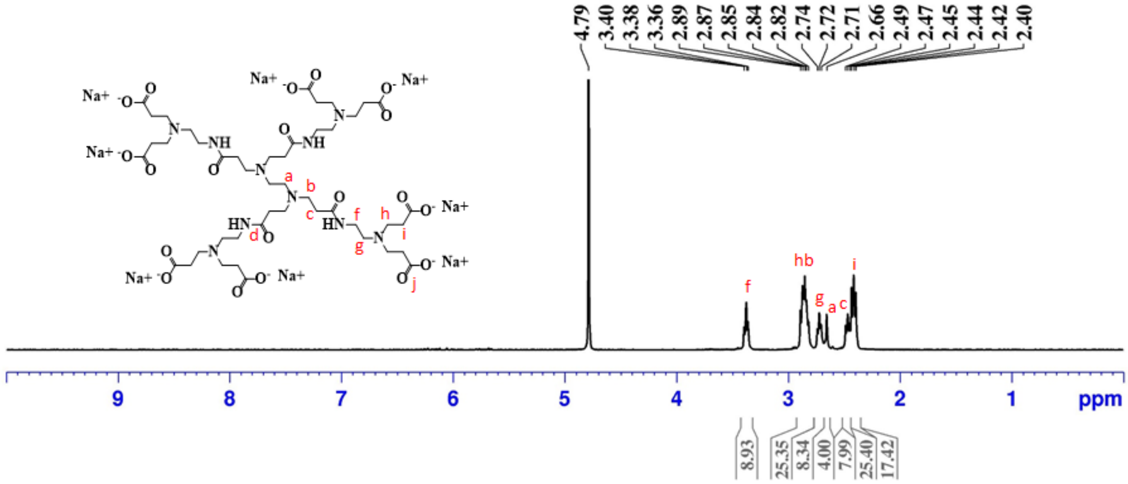



**Figure S3:** $^{1}H$-NMR spectrum of anionic PAMAM dendrimer G0.5$(COONa)_8$ in $D_2O$.

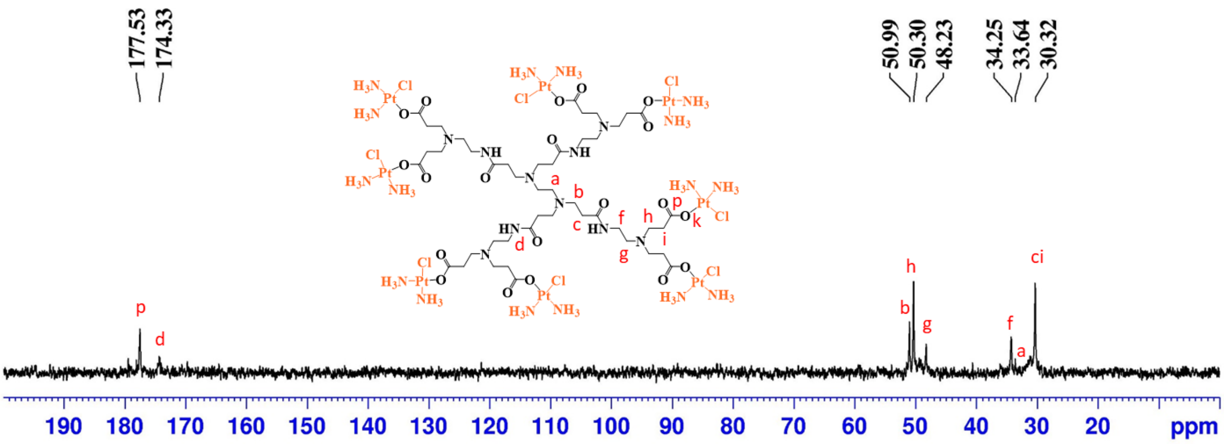



**Figure S4:** $^{13}C$-NMR spectrum of monodentate G0.5$(COOPt(NH_3)_2Cl)_8$ in $D_2O$.

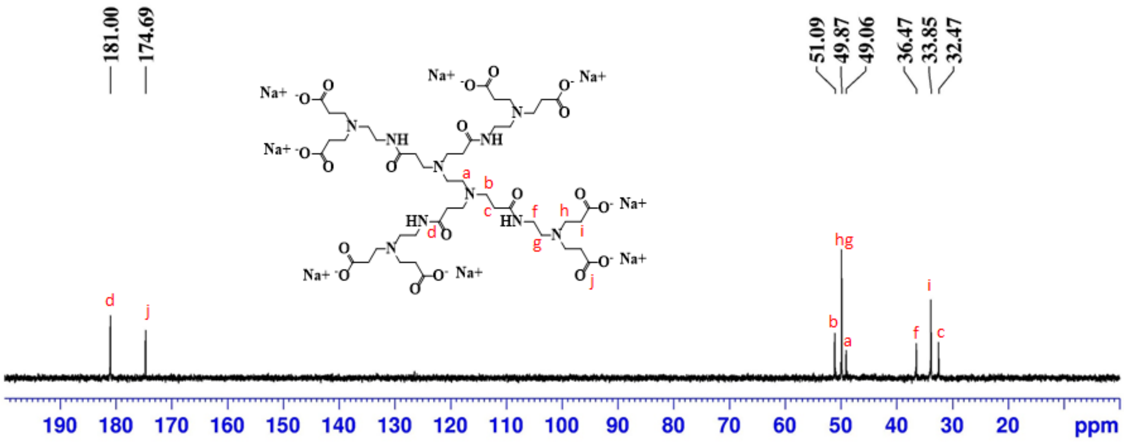



**Figure S5:** $^{13}C$-NMR spectrum of anionic PAMAM dendrimer G0.5$(COONa)_8$ in $D_2O$.

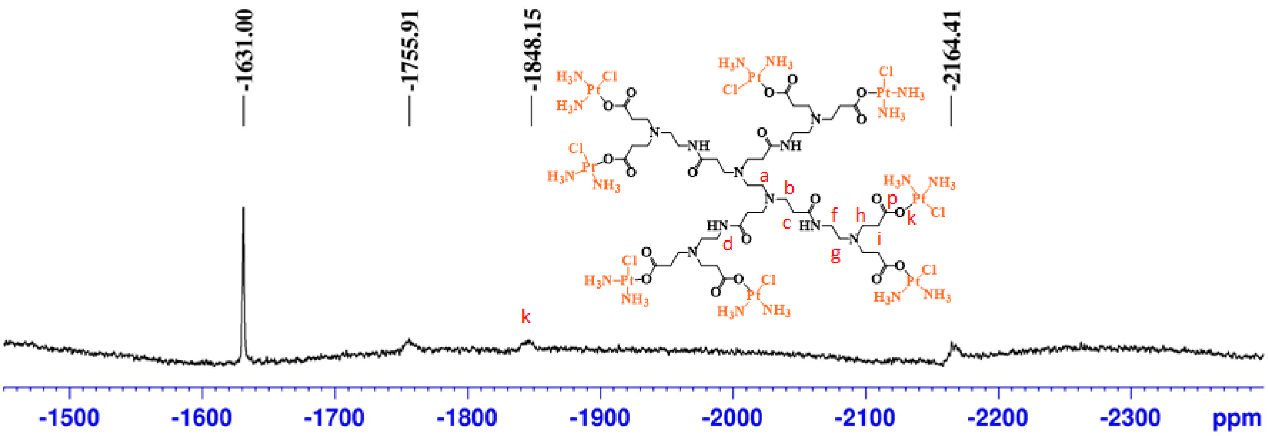



**Figure S6:** $^{195}$Pt-NMR spectrum of monodentate G0.5(COOPt($NH_3$)$_2$Cl)$_8$ in $D_2O$, with $K_2PtCl_4$ as external reference (-1631 ppm).

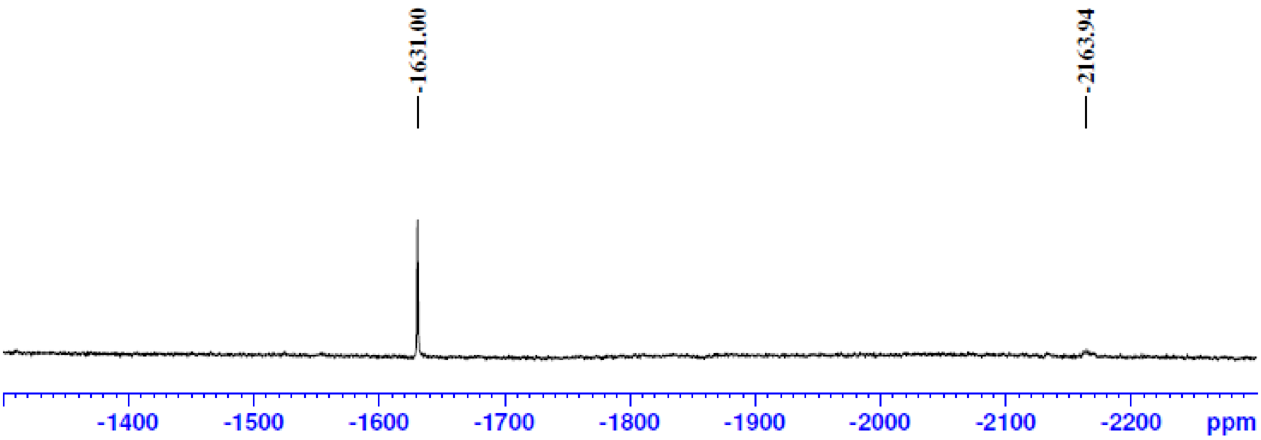



**Figure S7:** $^{195}$Pt-NMR spectrum of cisplatin in $D_2O$, with $K_2PtCl_4$ as external reference (-1631 ppm).

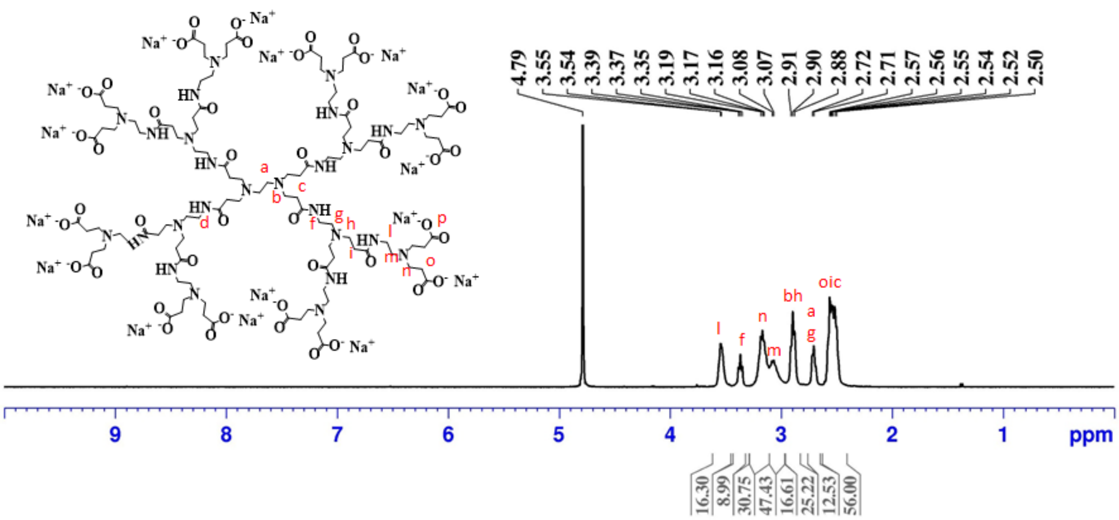



**Figure S8:** $^{1}$H-NMR spectrum of anionic PAMAM dendrimer G1.5(COONa)$_{16}$ in $D_2O$.

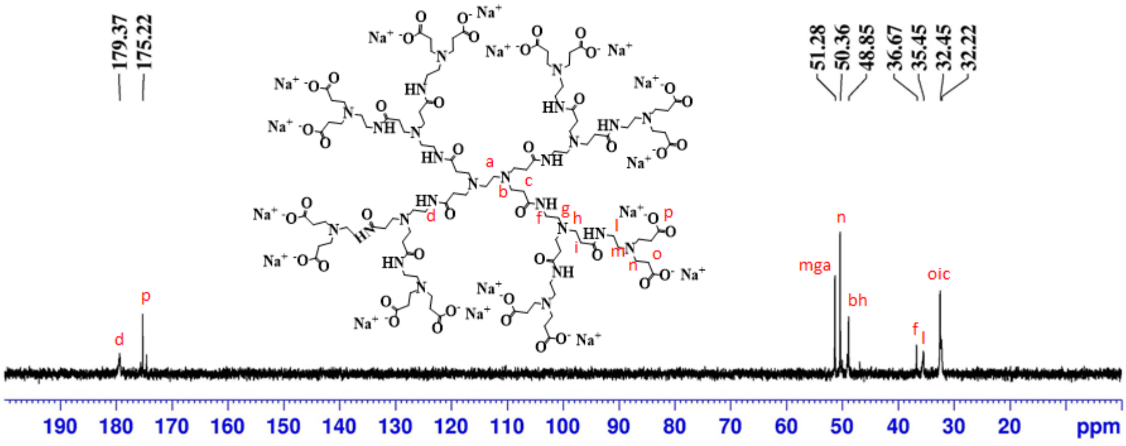



**Figure S9:** $^{13}C$ NMR spectrum of anionic PAMAM dendrimer G1.5$(COONa)_{16}$ in $D_2O$.

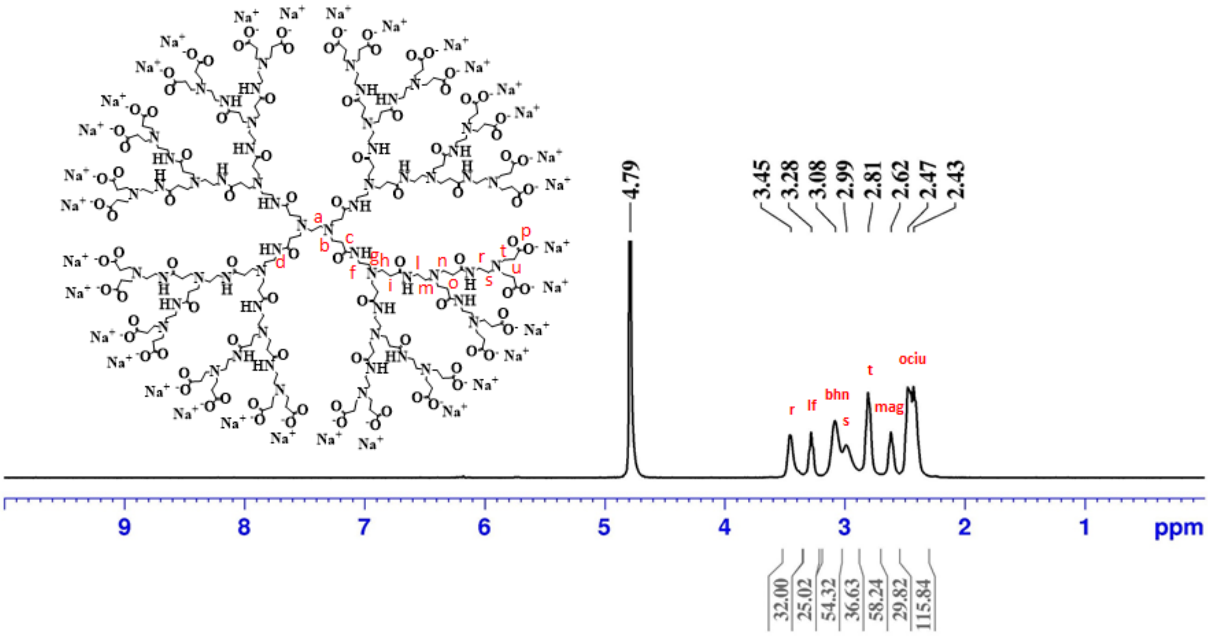



**Figure S10:** $^{1}H$-NMR spectrum of anionic PAMAM dendrimer G2.5$(COONa)_{32}$ in $D_2O$.

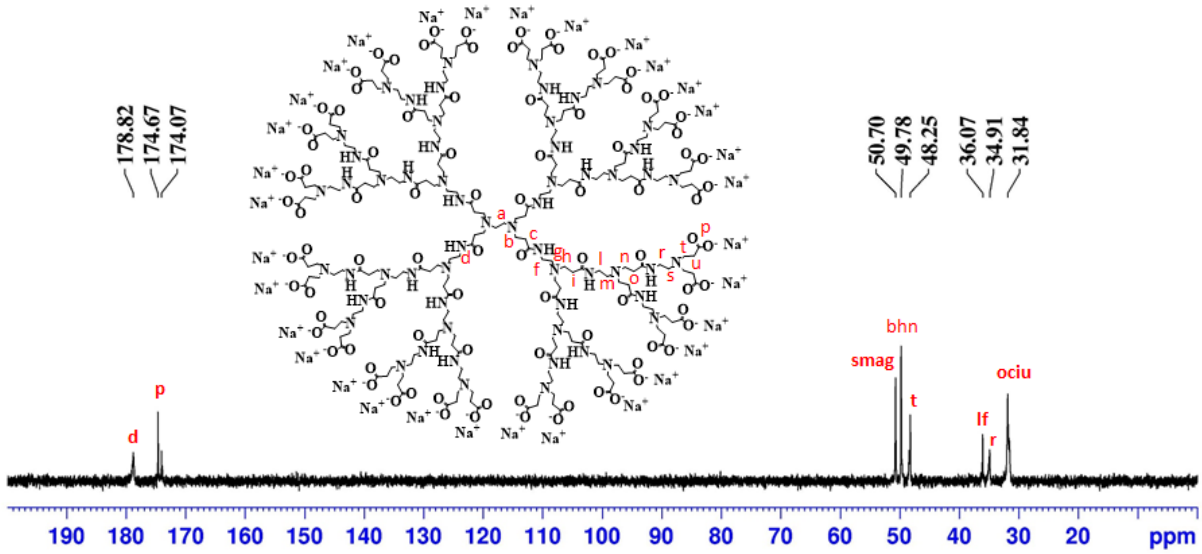



**Figure S11:** $^{13}$C-NMR spectrum of anionic PAMAM dendrimer G2.5(COONa)$_{32}$ in $D_2O$.

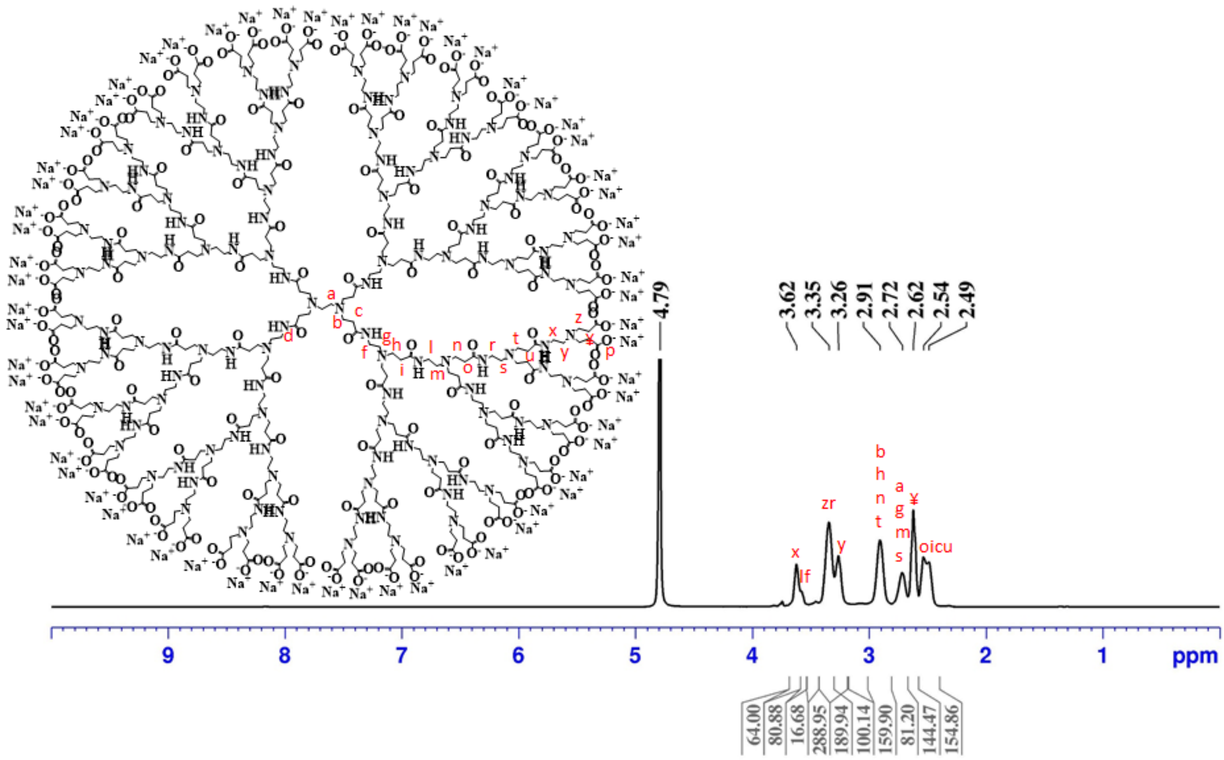



**Figure S12:** $^{1}$H-NMR spectrum of anionic PAMAM dendrimer G3.5(COONa)$_{64}$ in $D_2O$.

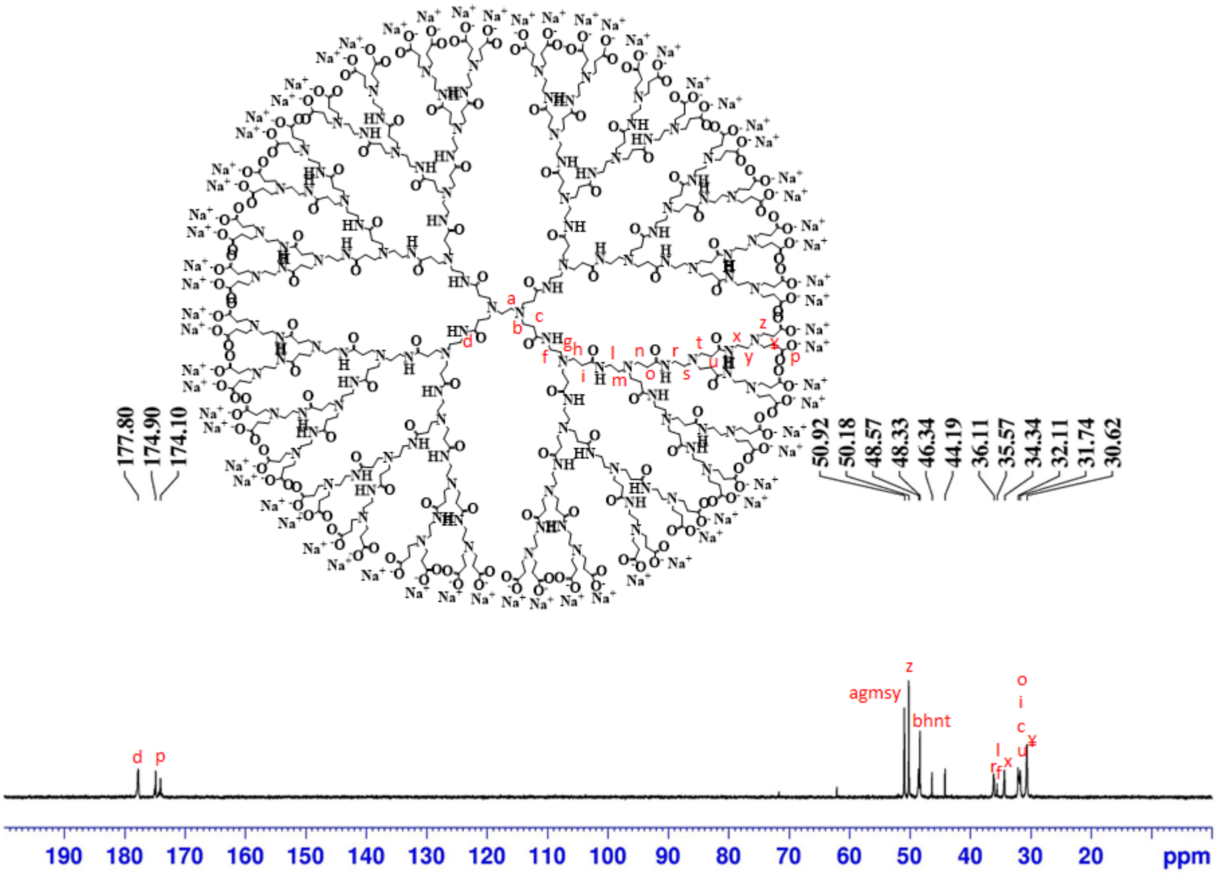


**Figure S13:** $^{13}$C-NMR spectrum of anionic PAMAM dendrimer G3.5$(COONa)_{64}$ in $D_2O$.

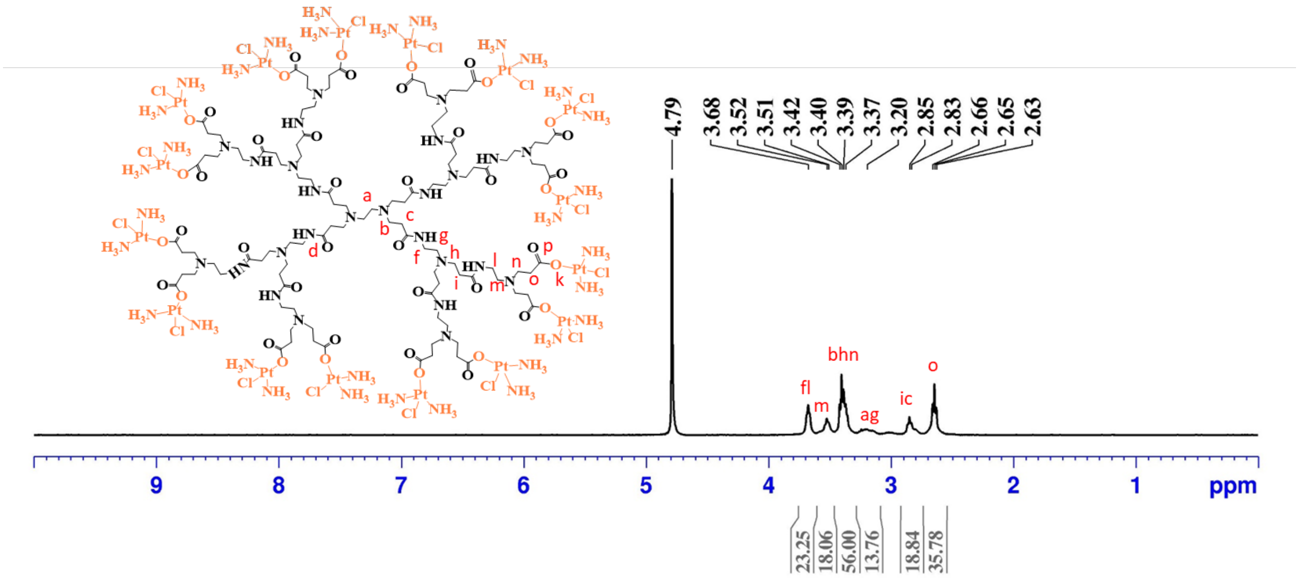


**Figure S14:** $^{1}$H-NMR spectrum of monodentate G1.5$(COOPt(NH_3)_2Cl)_{16}$ in $D_2O$.

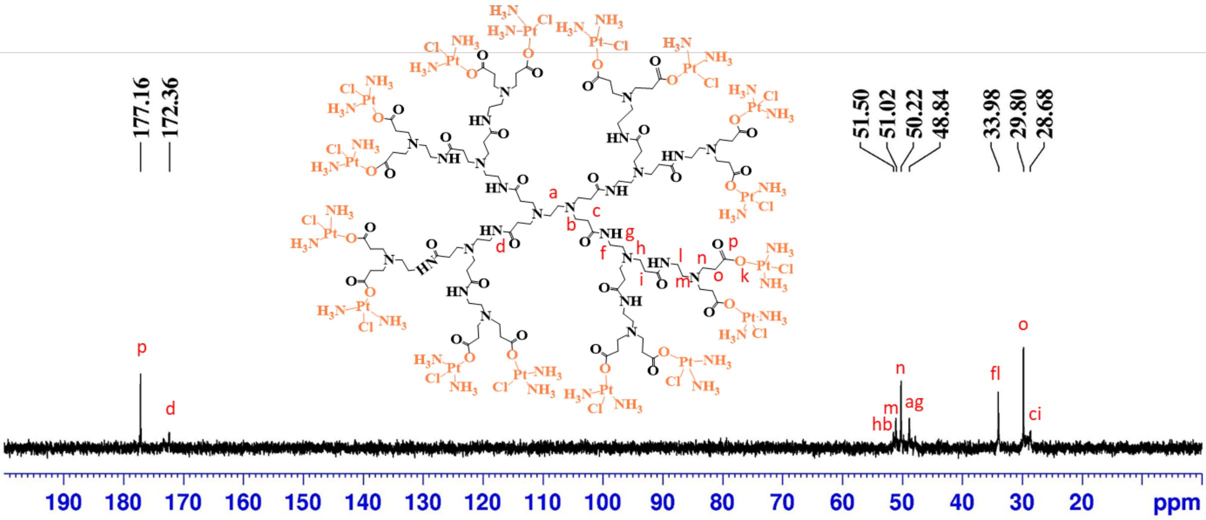



**Figure S15:** $^{13}C$ NMR spectrum of monodentate G1.5(COOPt($NH_3$)$_2$Cl)$_{16}$ in $D_2O$.

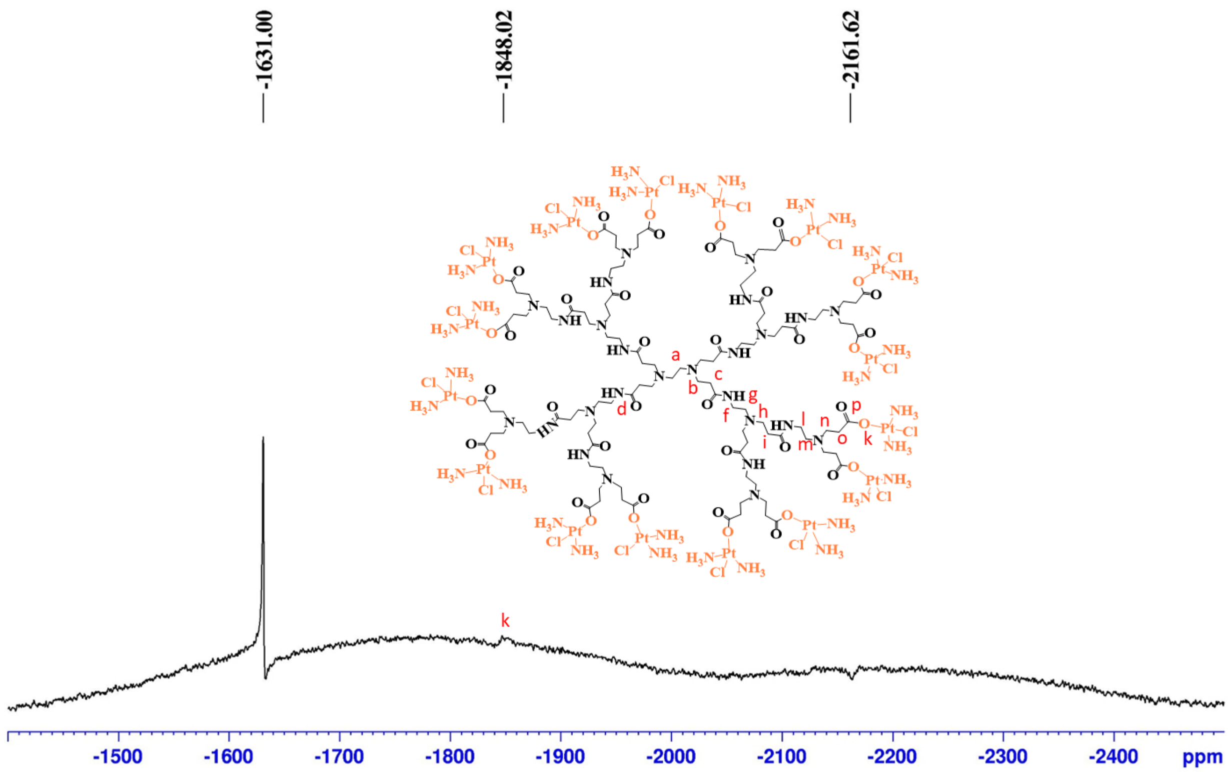



**Figure S16:** $^{195}Pt$-NMR spectrum of monodentate G1.5(COOPt($NH_3$)$_2$Cl)$_{16}$ in $D_2O$, with $K_2PtCl_4$ as an external reference (-1631 ppm).

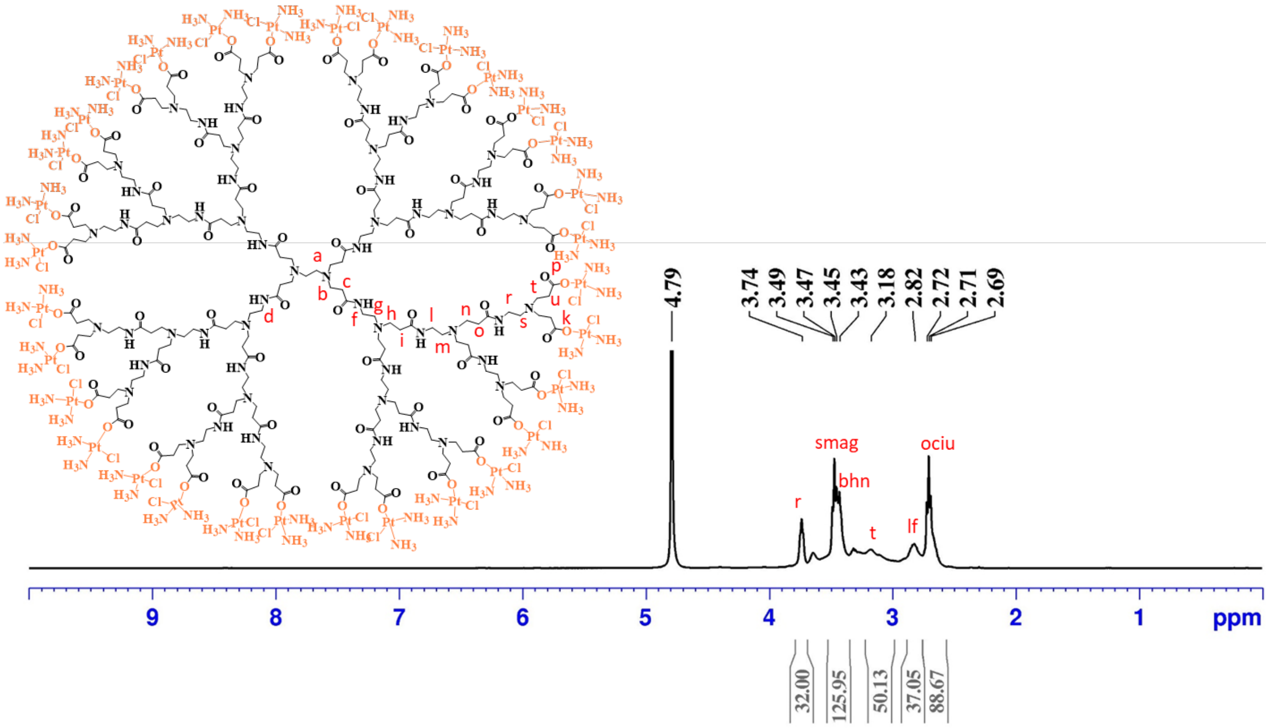



**Figure S17:** $^{1}$H-NMR spectrum of monodentate G2.5(COOPt(NH$_3$)$_2$Cl)$_{32}$in D$_2$O.

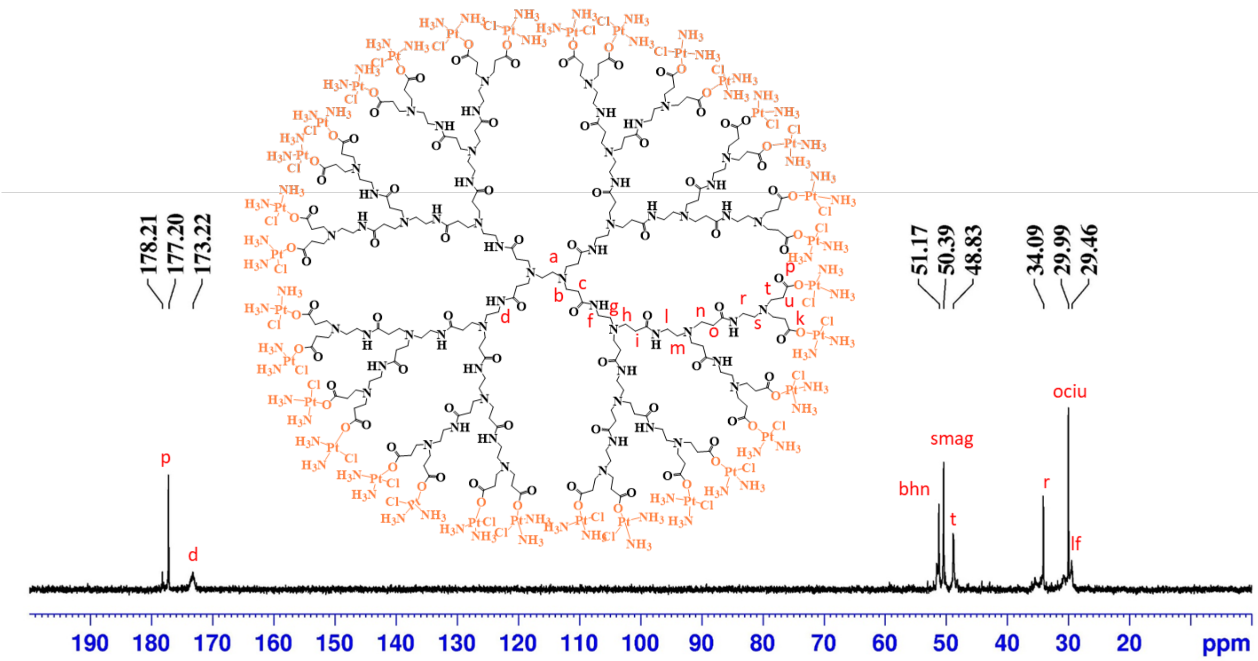



**Figure S18:** $^{13}$C-NMR spectrum of monodentate G2.5(COOPt(NH$_3$)$_2$Cl)$_{32}$ in D$_2$O.

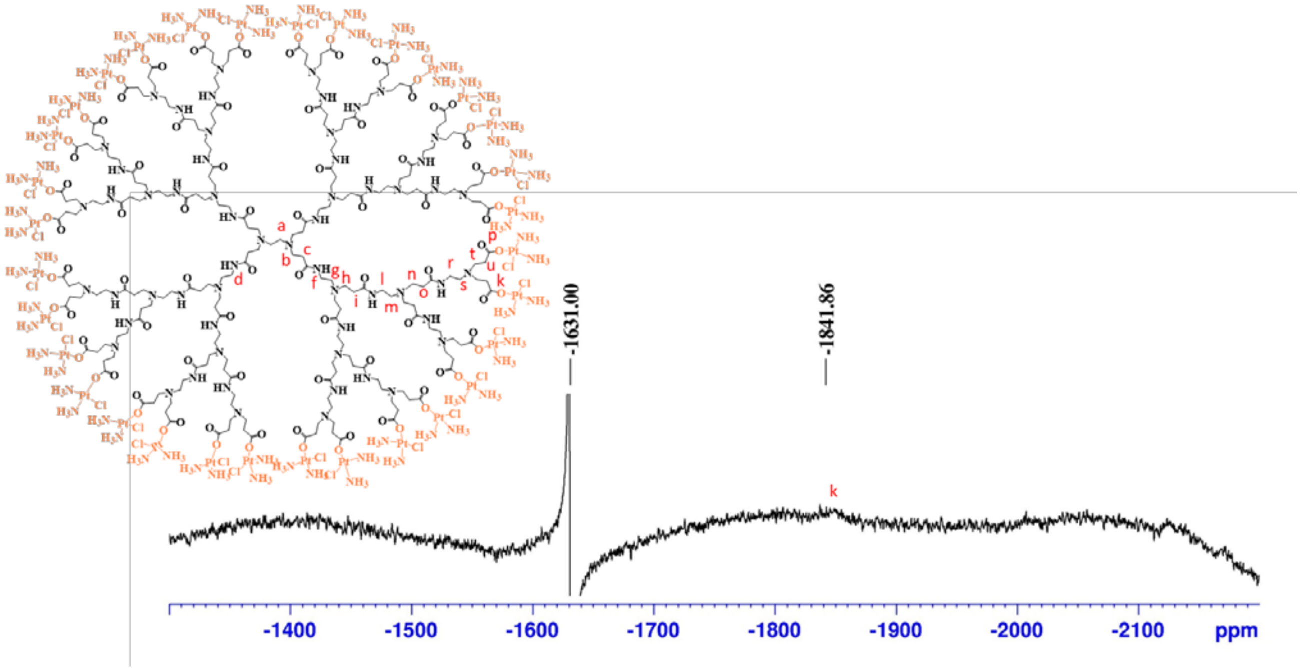



**Figure S19:** $^{195}Pt$-NMR spectrum of monodentate G2.5($COOPt(NH_3)_2Cl)_{32}$ in $D_2O$, with $K_2PtCl_4$ as an external reference (-1631 ppm).

**Table S1:** Molecular weight of the cisplatin-metallodendrimers in a monodentate form.

| | **G0.5($COOPt(NH_3)_2Cl)_8$** | **G1.5($COOPt(NH_3)_2Cl)_{16}$** | **G2.5($COOPt(NH_3)_2Cl)_{32}$** |
|---|---|---|---|
| **Molecular weight** | 3201.90 | 6800.29 | 13997.08 |
| **m/z calculated** | 1606.68 | 1013.47 | 974.82 |
| **m/z found** | 1606.46<br>$[M+2H^{+}+ H_2O]^{2+}$<br><br>$C_{46}H_{118}Cl_8N_{26}O_{21}Pt_8^{2+}$ | 1013.46<br>$[M+3H^{+}]^{3+}$<br><br>$C_{110}H_{205}N_{30}O_{44}Pt_2^{\ 3+}$ | 974.65<br>$[M+H^{+}]^{+}$<br><br>$C_{238}H_{562}Cl_{29}N_{116}O_{92}Pt_{29}^{+}$ |

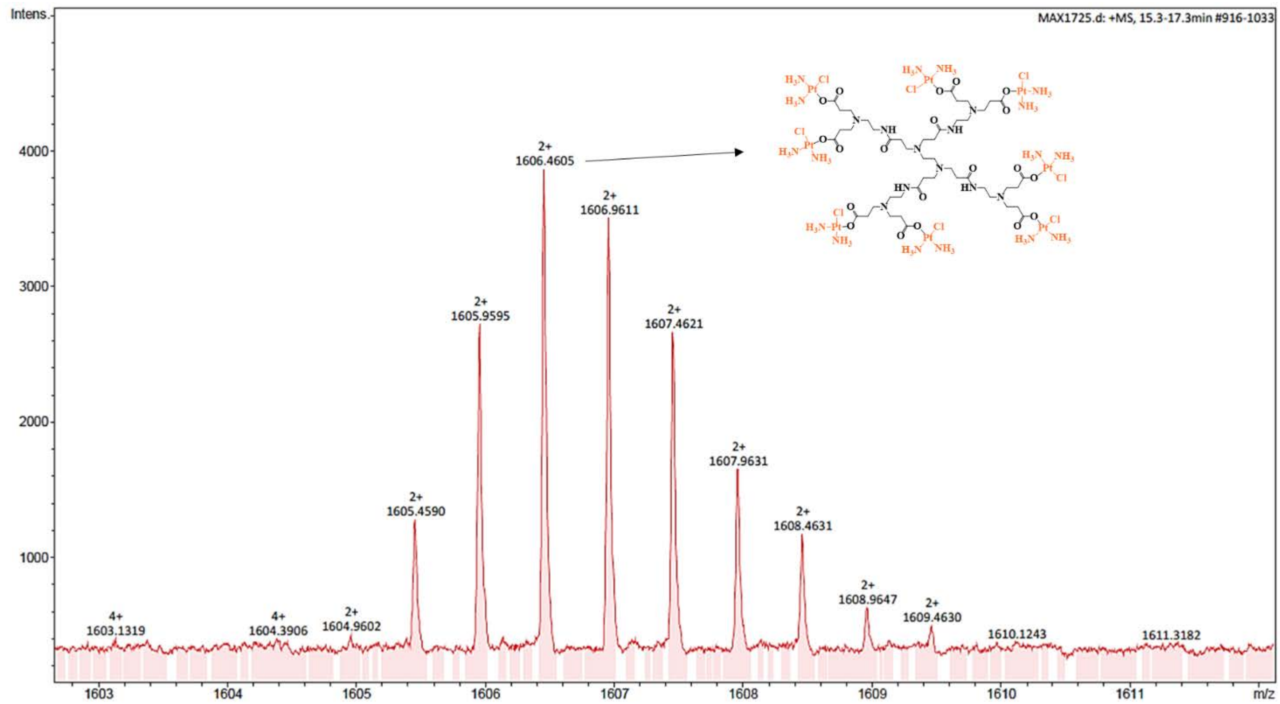


**Figure S20:** TOF-MS (ESI+) mass spectrum of monodentate G0.5(COOPt($NH_3$)$_2$Cl)$_8$ metallodendrimer.

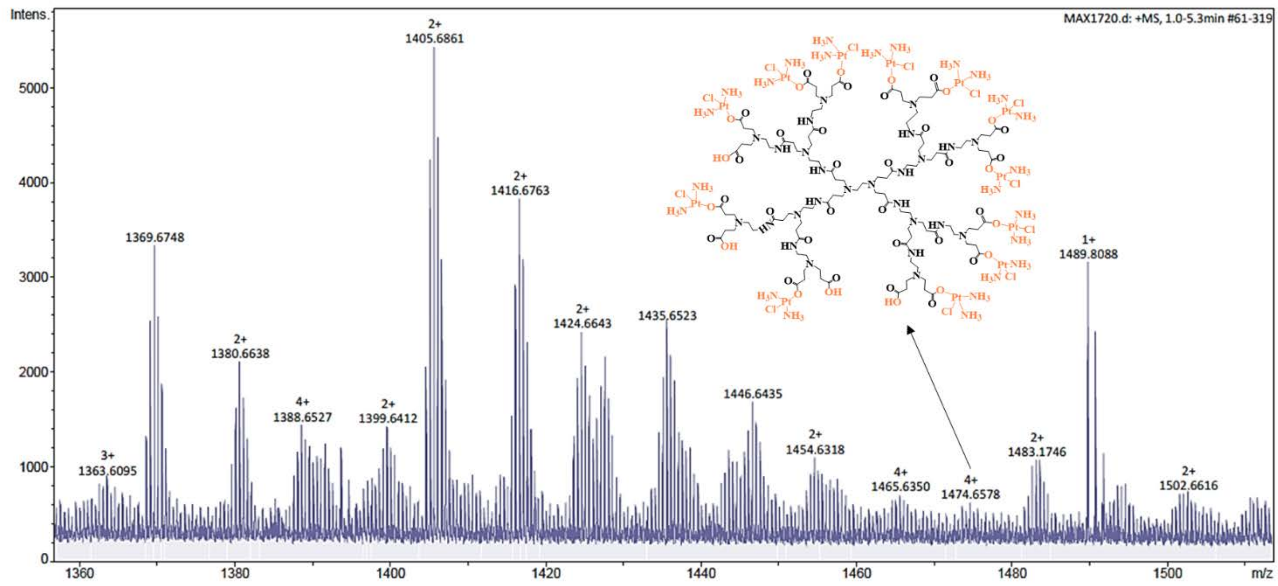


**Figure S21:** TOF-MS (ESI+) mass spectrum of monodentate G1.5(COOPt($NH_3$)$_2$Cl)$_{16}$ metallodendrimer.

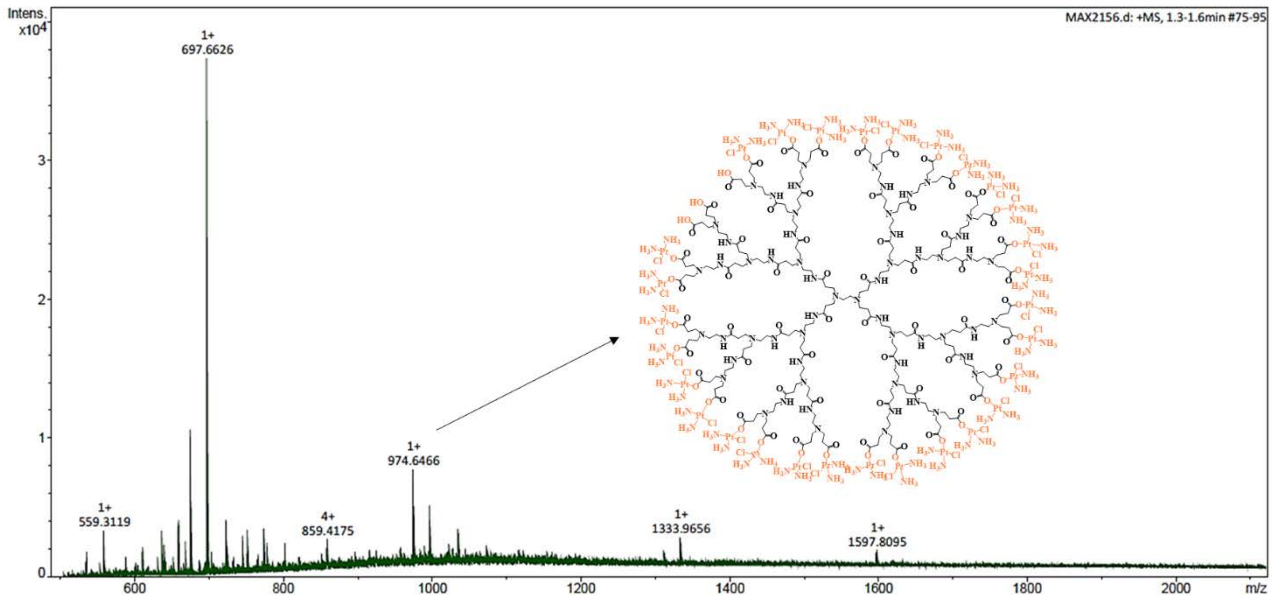



**Figure S22:** TOF-MS (ESI+) mass spectrum of monodentate G2.5(COOPt($NH_3$)$_2$Cl)$_{32}$ metallodendrimer.

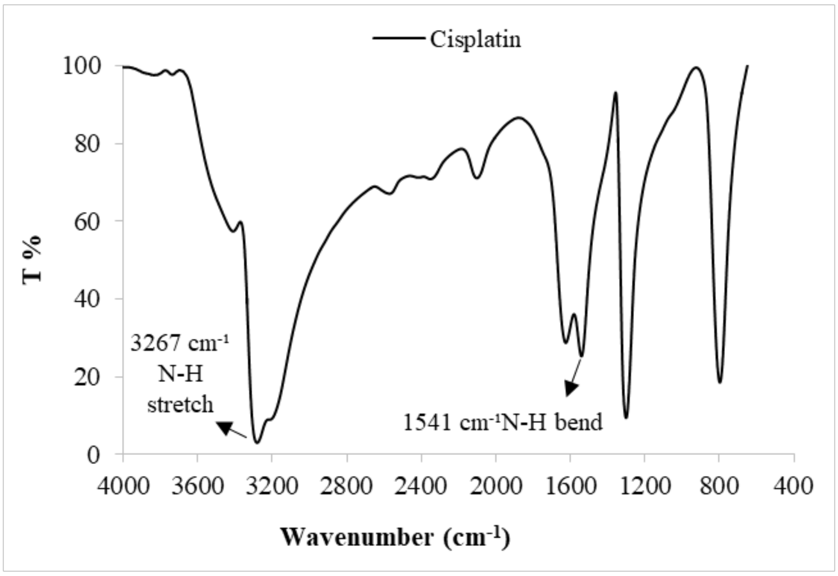



**Figure S23:** FTIR spectra of cisplatin in KBr pellet.

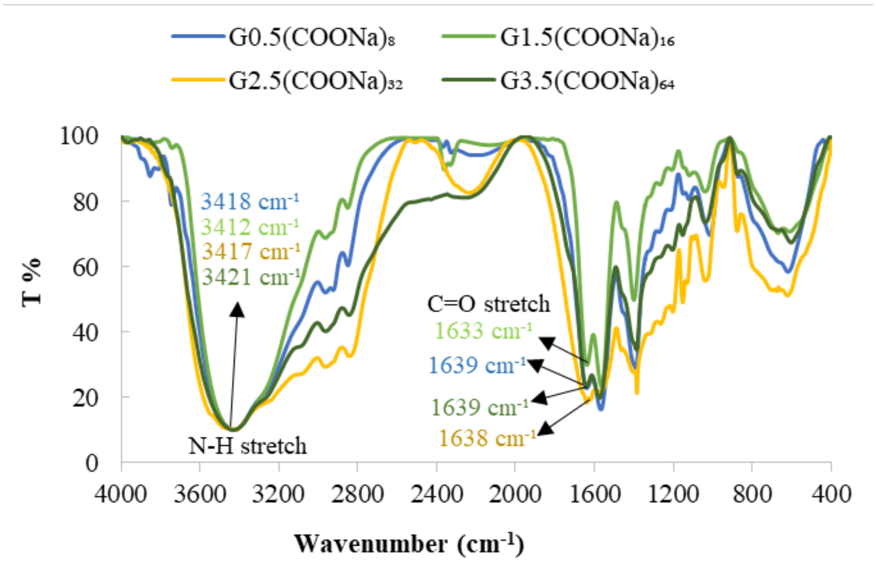



**Figure S24:** FTIR spectra of different generations of anionic PAMAM dendrimers (G0.5-G3.5) in KBr pellet.

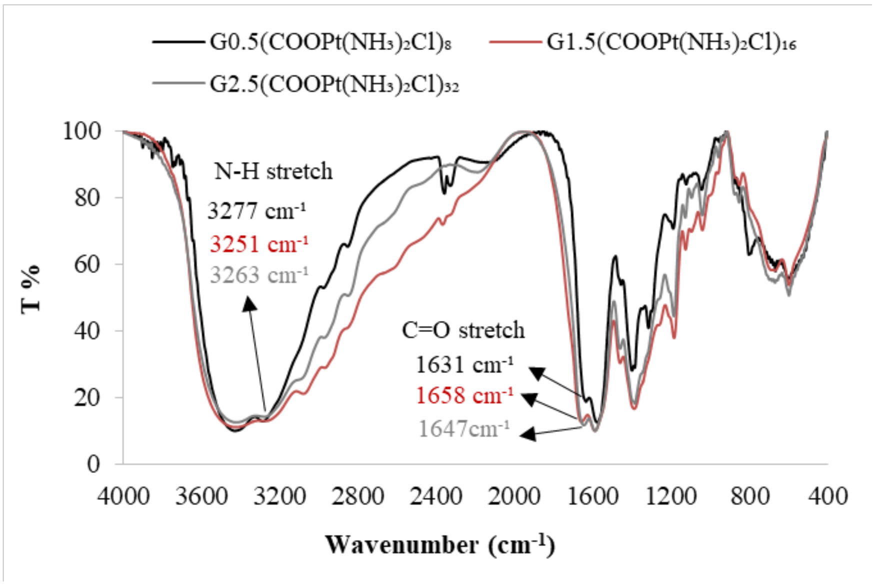
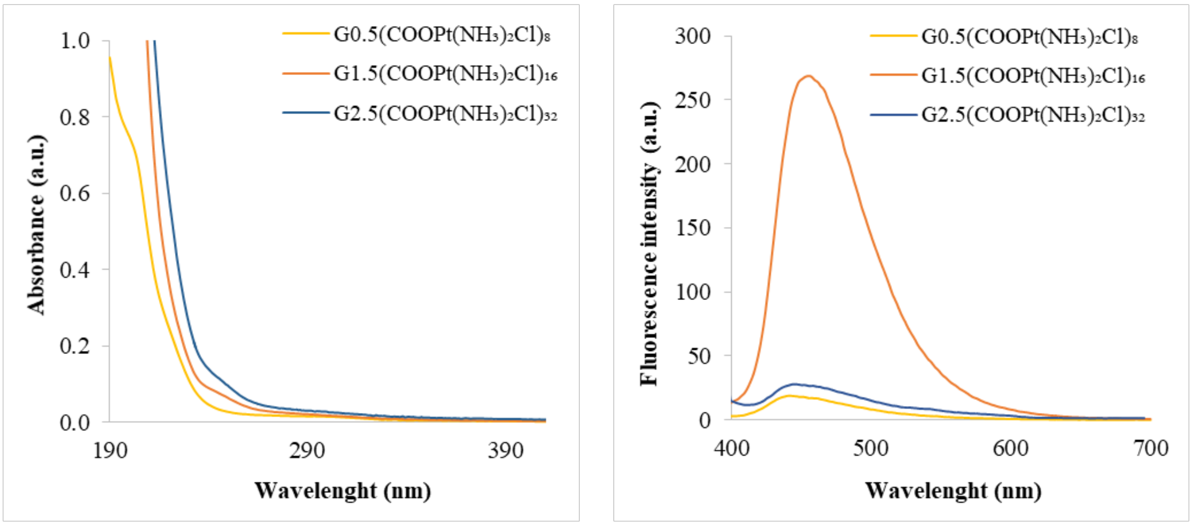
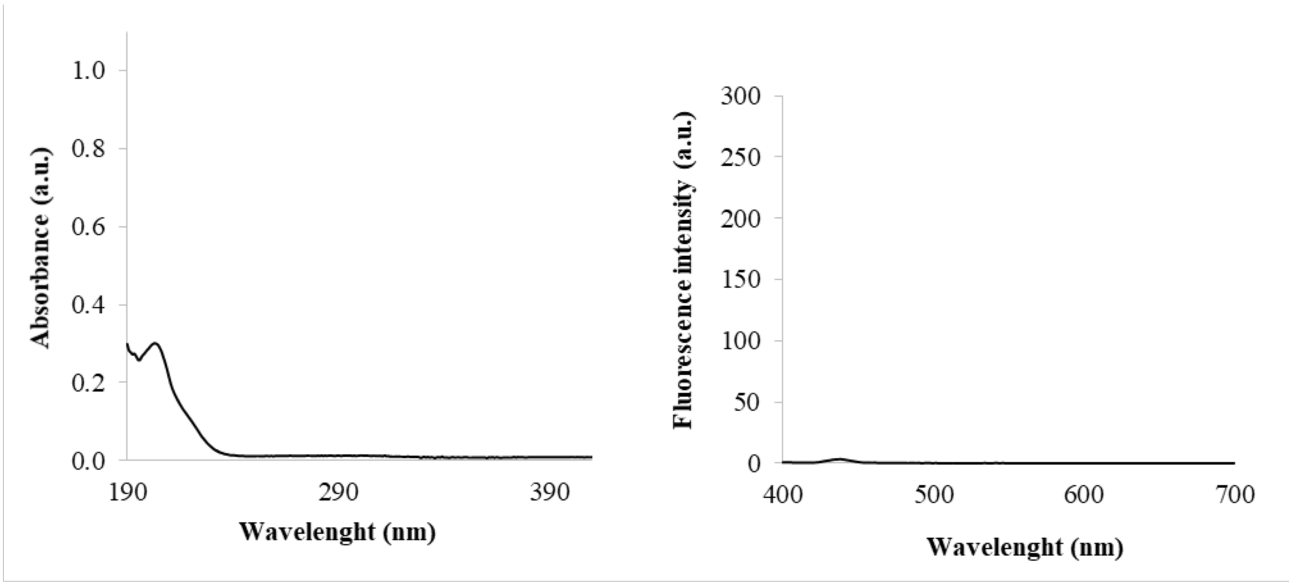



**Figure S25:** FTIR spectra of metallodendrimers conjugated with cisplatin in monodentate form. The spectra were performed in KBr pellet.




(a) (b)

**Figure S26:** a) Absorption spectra of cisplatin-metallodendrimers in the monodentate form recorded at a concentration of 40 μM in ultrapure water and b) Emission ($\lambda_{ex}$ = 380 nm) spectra of cisplatin-metallodendrimers in the monodentate form recorded at a concentration of 500 μM in ultrapure water.




(a) (b)

**Figure S27:** a) Absorption spectra of cisplatin recorded at a concentration of 40 μM of in ultrapure water and b) Emission ($\lambda_{ex}$ = 380 nm) spectra of cisplatin recorded at a concentration of 500 μM in ultrapure water.

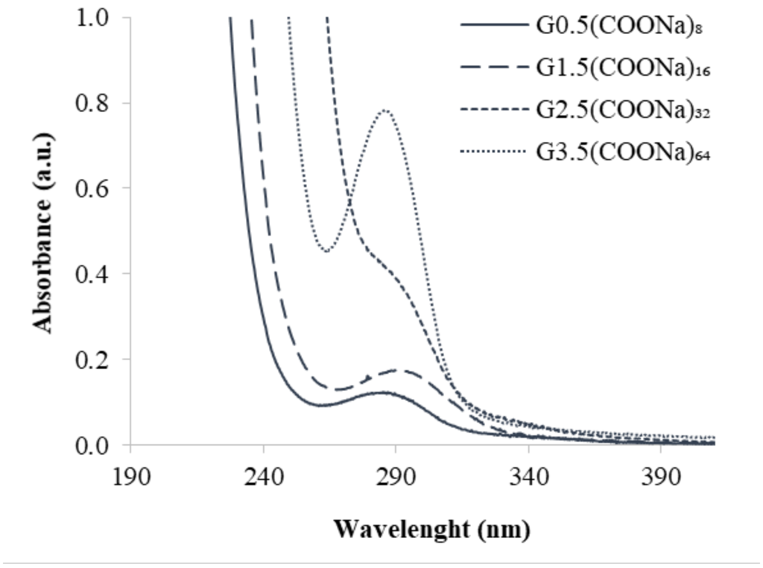



**Figure S28:** UV-Vis spectra of anionic PAMAM dendrimers at a concentration of 500 μM in ultrapure water.

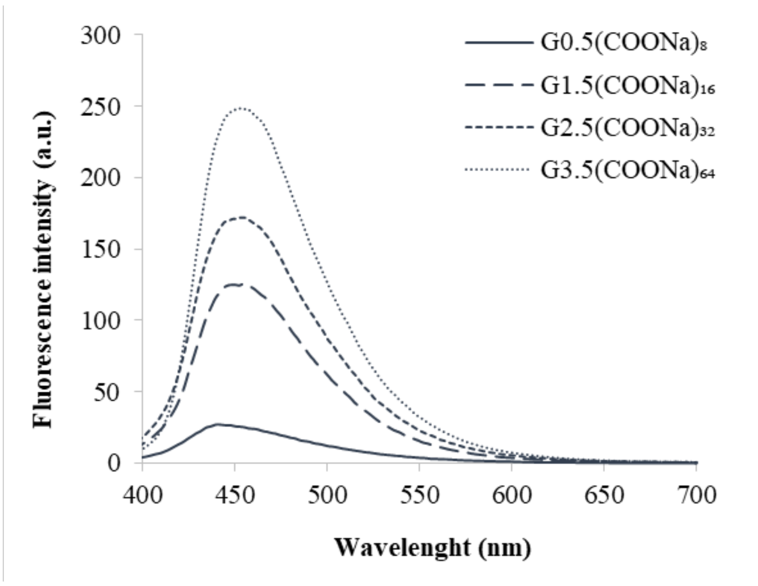



**Figure S29:** Emission ($\lambda_{ex}$= 380 nm) of anionic PAMAM dendrimers at a concentration of 500 μM in ultrapure water.

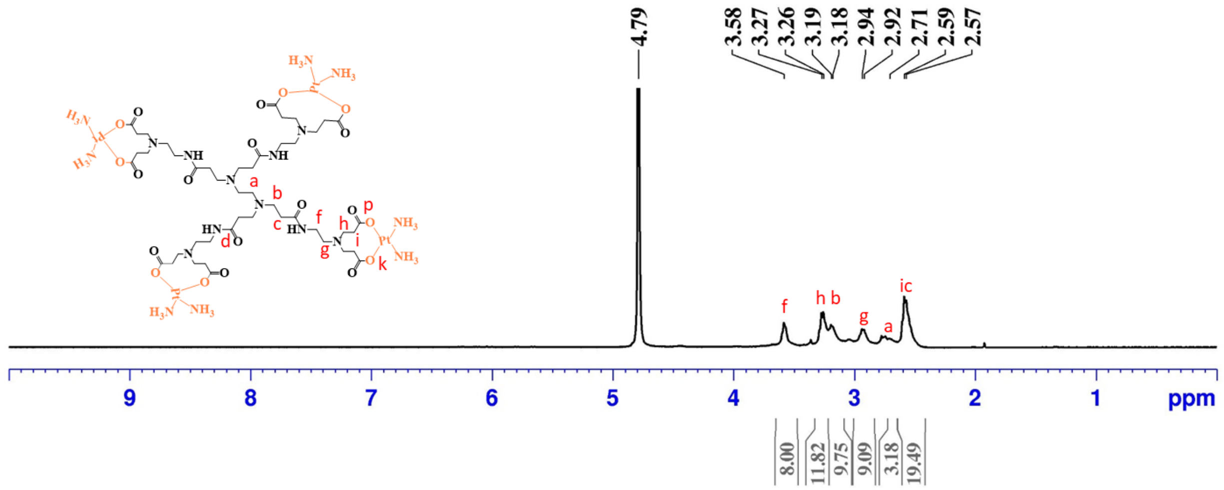



**Figure S30:** [1]H-NMR spectrum of bidentate G0.5(COOPt$(NH_3)_2)_4$ in $D_2O$.

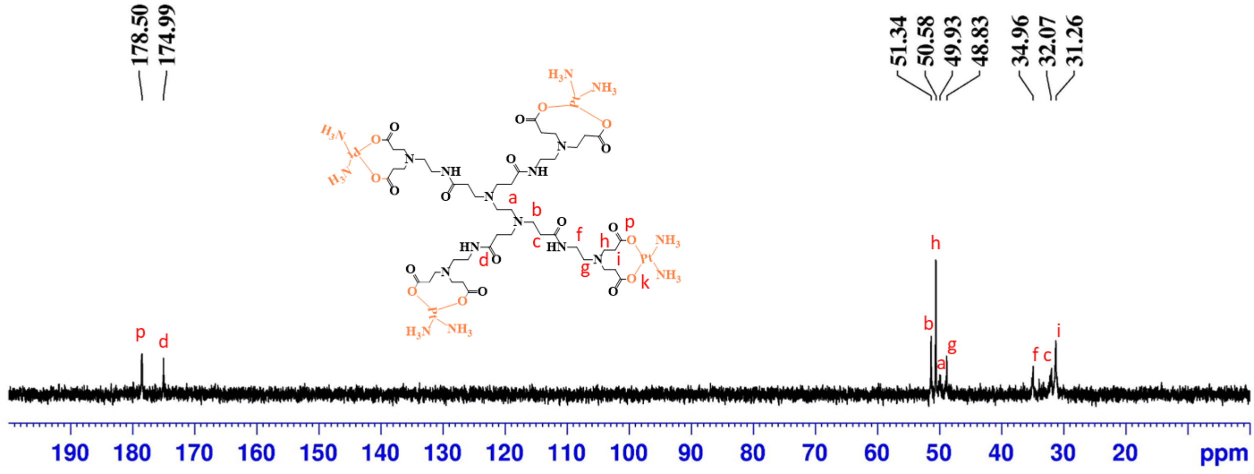



**Figure S31:** [13]C-NMR spectrum of bidentate G0.5(COOPt$(NH_3)_2)_4$ in $D_2O$.

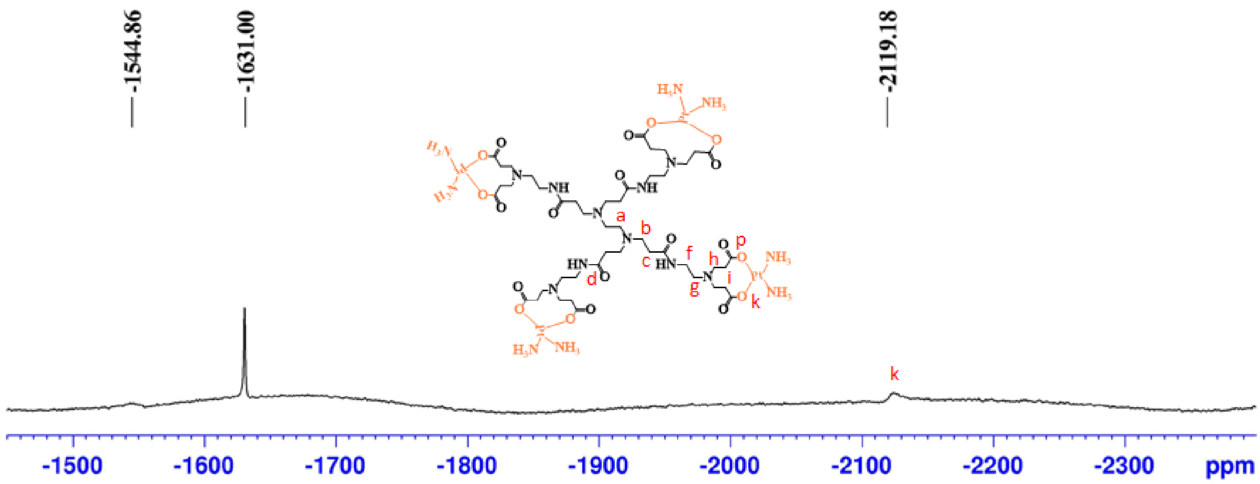


**Figure S32:** $^{195}$Pt-NMR spectrum of bidentate G0.5COO(Pt(NH$_3$)$_2$)$_4$ in $D_2O$, with $K_2PtCl_4$ as external reference (-1631 ppm).

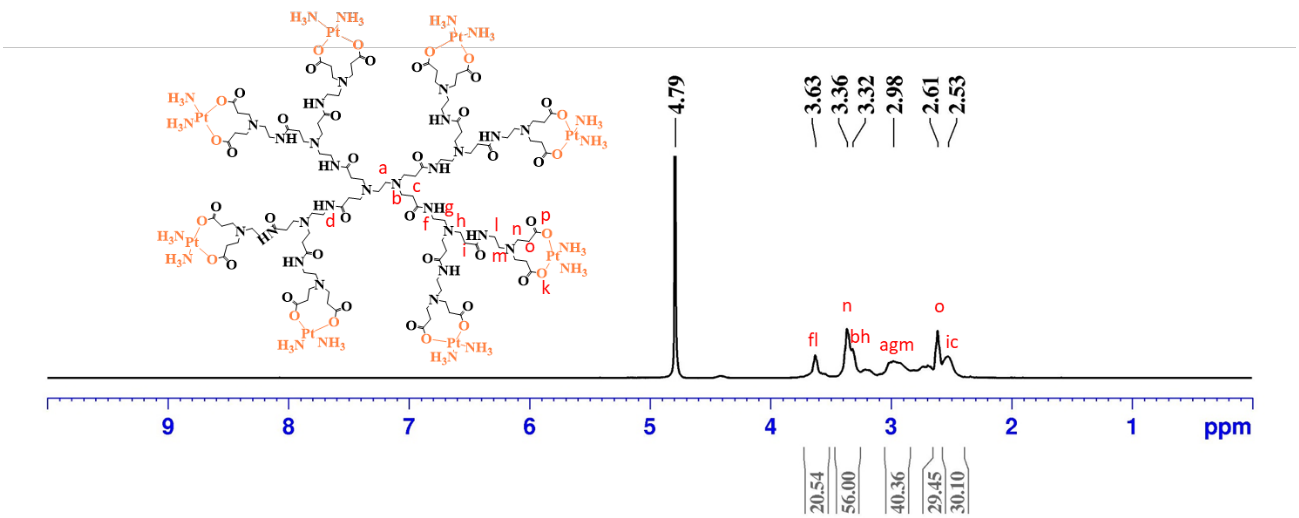


**Figure S33:** $^{1}$H-NMR spectrum of bidentate G1.5COO(Pt(NH$_3$)$_2$)$_8$ in $D_2O$.

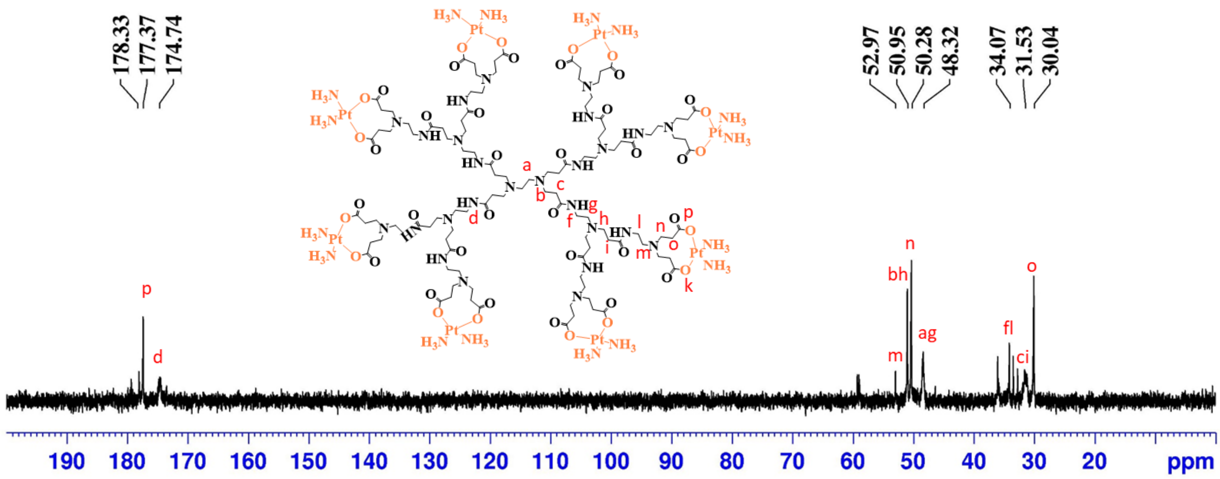



**Figure S34:** $^{13}C$-NMR spectrum of bidentate G1.5COOPt($NH_3$)$_2$)$_8$ in $D_2O$.

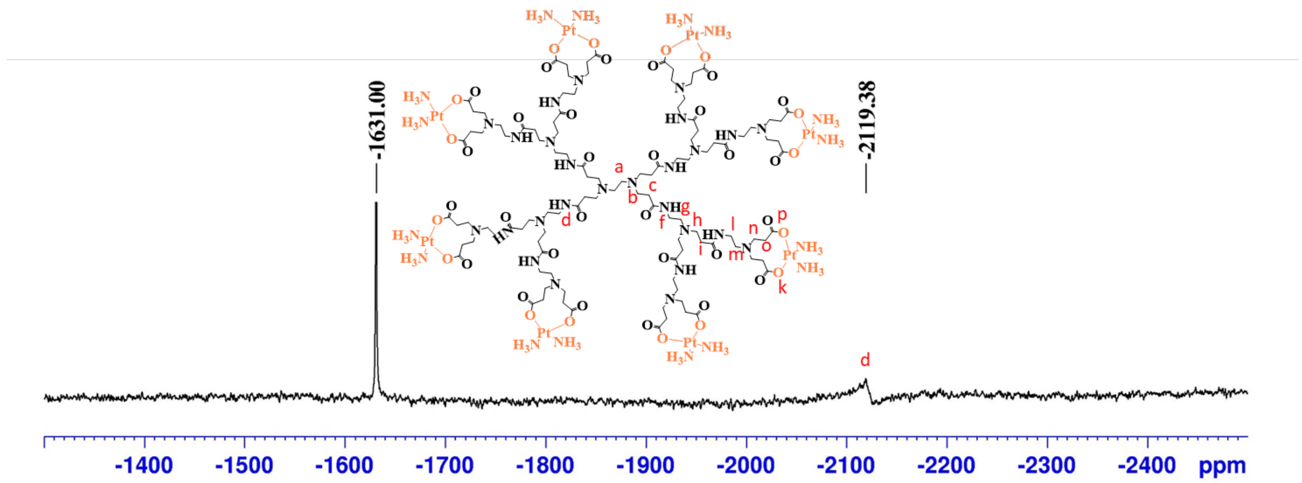



**Figure S35:** $^{195}Pt$-NMR spectrum of bidentate G1.5COO(Pt($NH_3$)$_2$)$_8$ in $D_2O$, with $K_2PtCl_4$ as external reference (-1631 ppm).

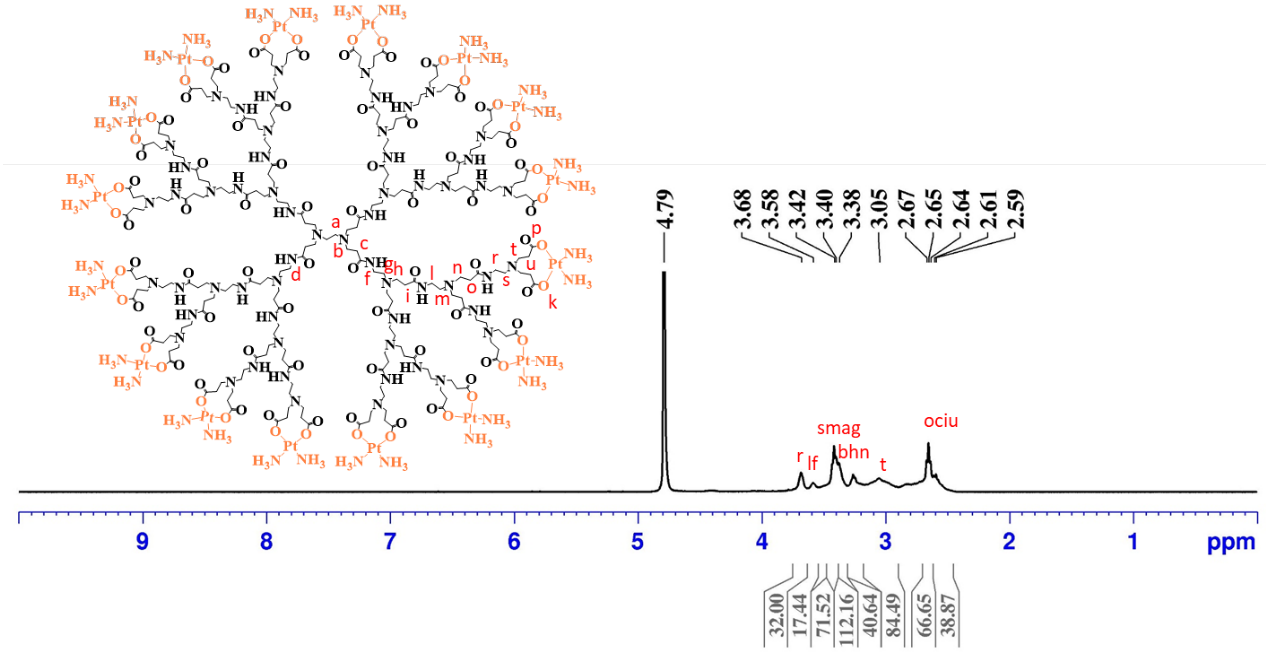



**Figure S36:** [1]H-NMR spectrum of bidentate G2.5COO(Pt(NH3)2)16 in D2O.

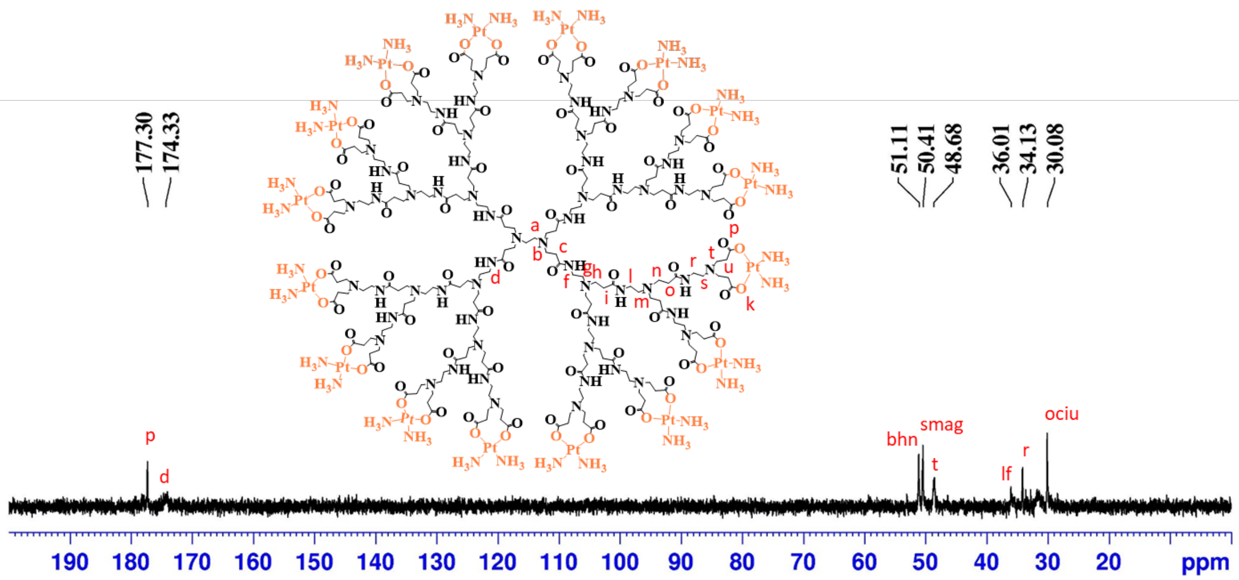



**Figure S37:** [13]C-NMR spectrum of bidentate G2.5COO(Pt(NH3)2)16 in D2O.

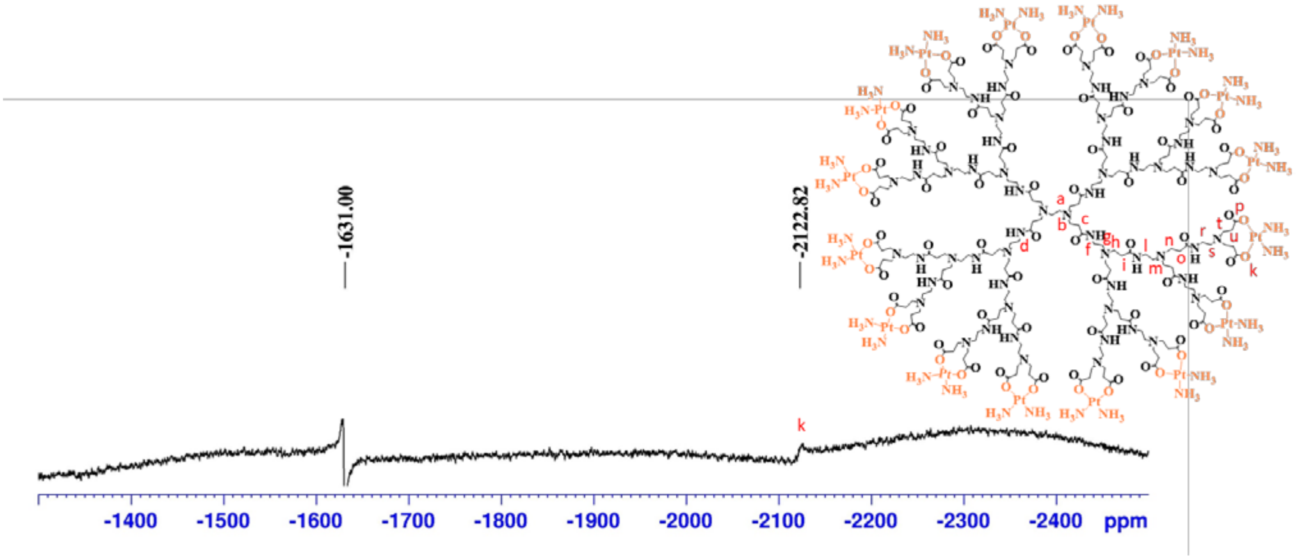



**Figure S38:** $^{195}$Pt-NMR spectrum of bidentate G2.5COO(Pt($NH_3$)$_2$)$_{16}$ in $D_2O$, with $K_2PtCl_4$ as external reference (-1631 ppm).

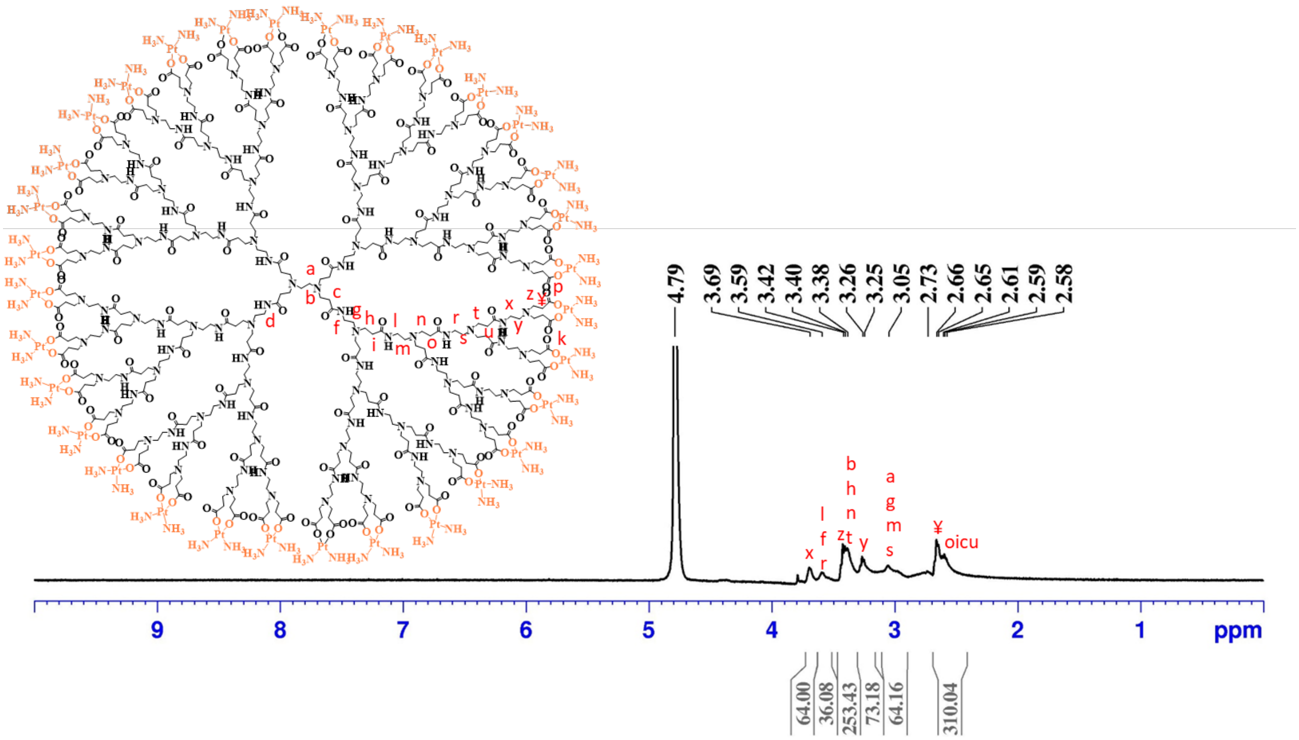



**Figure S39:** $^{1}$H-NMR spectrum of bidentate G3.5COO(Pt($NH_3$)$_2$)$_{32}$ in $D_2O$.

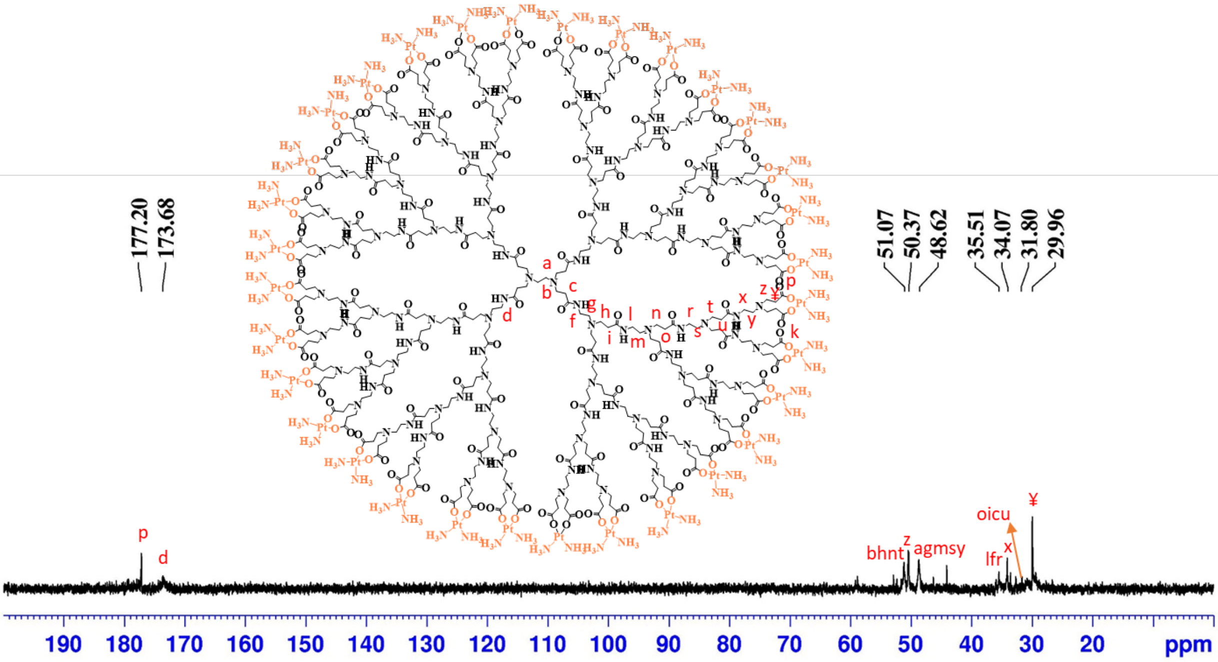



**Figure S40:** $^{13}C$-NMR spectrum of bidentate G3.5COO$(Pt(NH_3)_2)_{32}$ in $D_2O$.

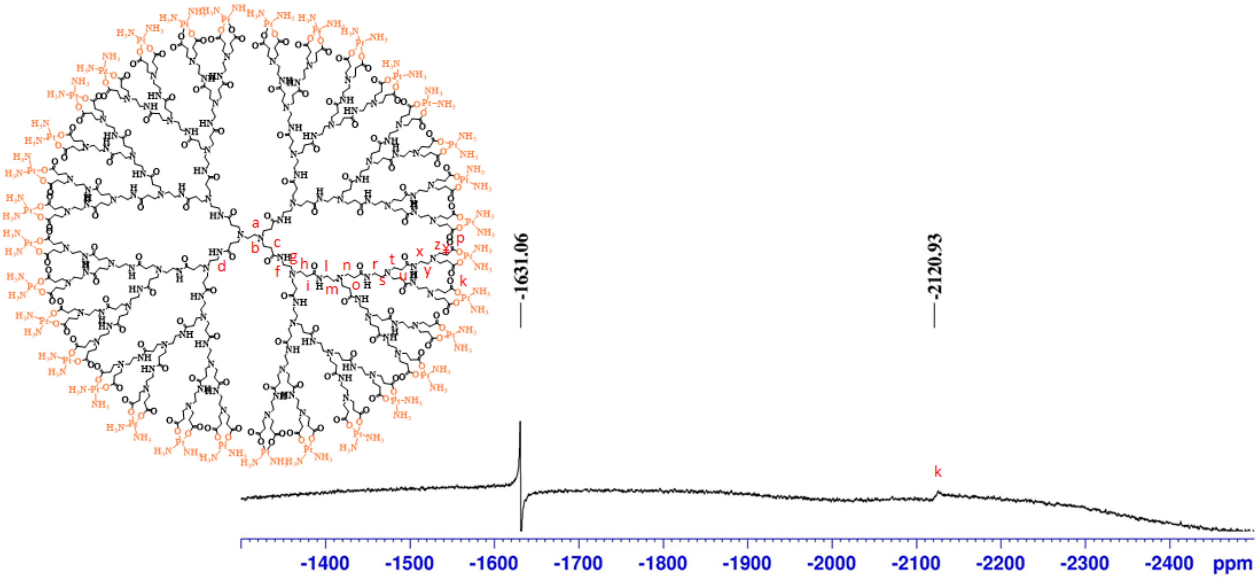



**Figure S41:** $^{195}Pt$-NMR spectrum of bidentate G3.5COO$(Pt(NH_3)_2)_{32}$ in $D_2O$, with $K_2PtCl_4$ as external reference (-1631 ppm).

**Table S2:** Molecular weight of the cisplatin-metallodendrimers in a bidentate form.

| | **$G0.5COO(Pt(NH_3)_2)_4$** | **$G1.5COO(Pt(NH_3)_2)_8$** | **$G2.5COO(Pt(NH_3)_2)_{16}$** | **$G3.5COO(Pt(NH_3)_2)_{32}$** |
|---|---|---|---|---|
| **Molecular weight** | 2001.72 | 4399.92 | 9196.34 | 18789.17 |
| **m/z calculated** | 1023.27 | 1466.80 | 4598.51 | 974.82 |
| **m/z found** | 1023.27 $[M+2Na^+]^{2+}$ | 1466.83 $[M+3H^+]^{3+}$ | 4597.5 $[M+2H^+]^{2+}$ | 974.64 $[M+H^+]^+$ |
| | $C_{46}H_{96}N_{18}Na_2O_{20}Pt_4^{2+}$ | $C_{110}H_{227}N_{42}O_{44}Pt_8^{3+}$ | $C_{238}H_{482}N_{90}O_{92}Pt_{16}^{2+}$ | $C_{494}H_{981}N_{180}O_{188}Pt_{29}^{+}$ |

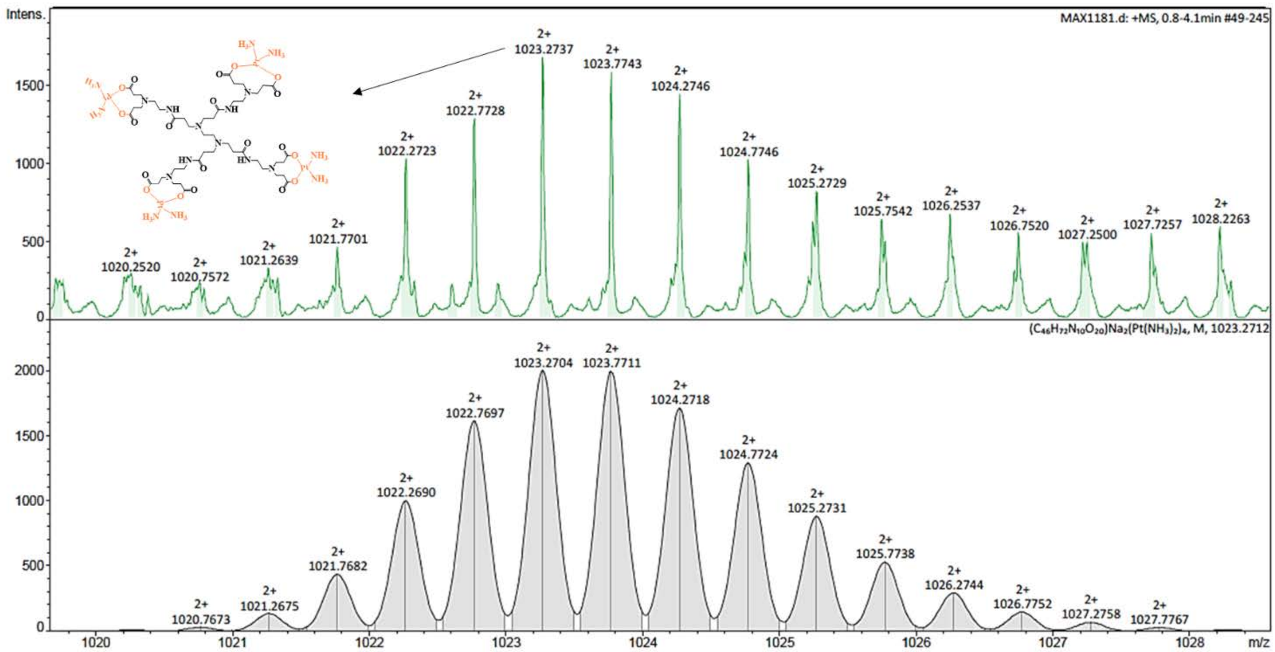



**Figure S42:** TOF-MS (ESI+) mass spectrum of bidentate $G0.5COO(Pt(NH_3)_2)_4$ metallodendrimer.

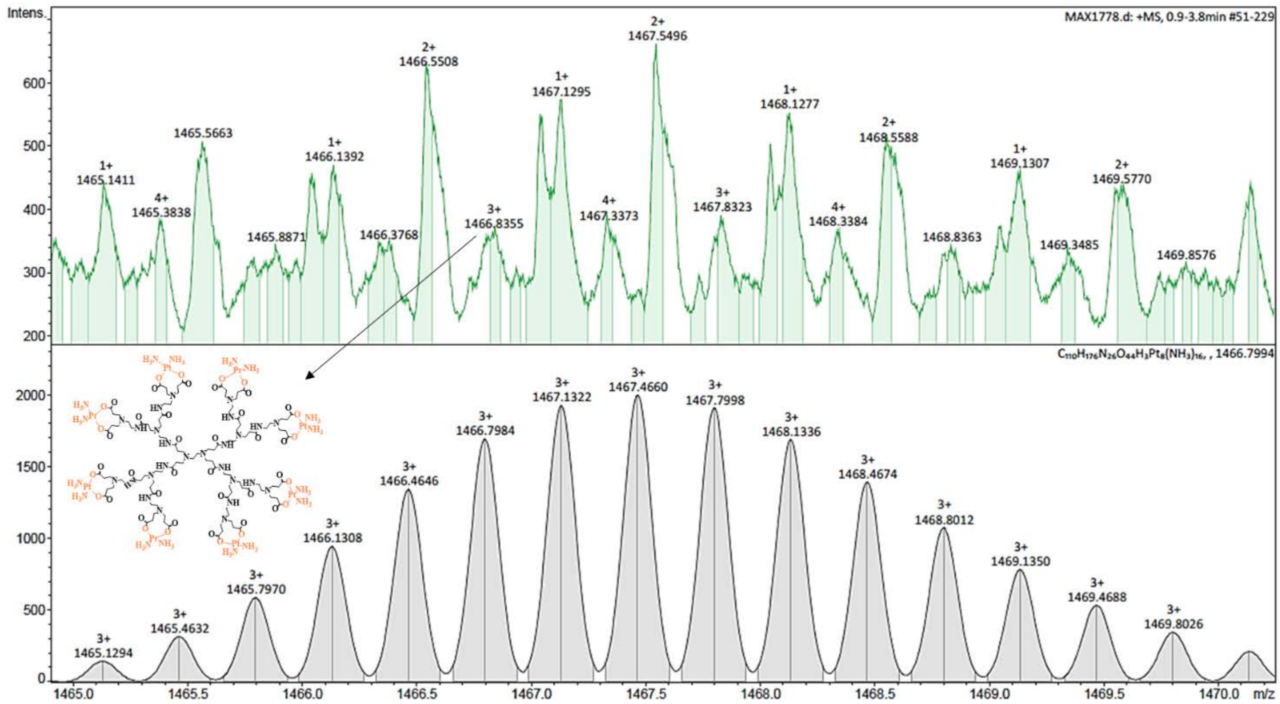



**Figure S43:** TOF-MS (ESI+) mass spectrum of bidentate G1.5COO(Pt(NH$_3$)$_2$)$_8$ metallodendrimer.

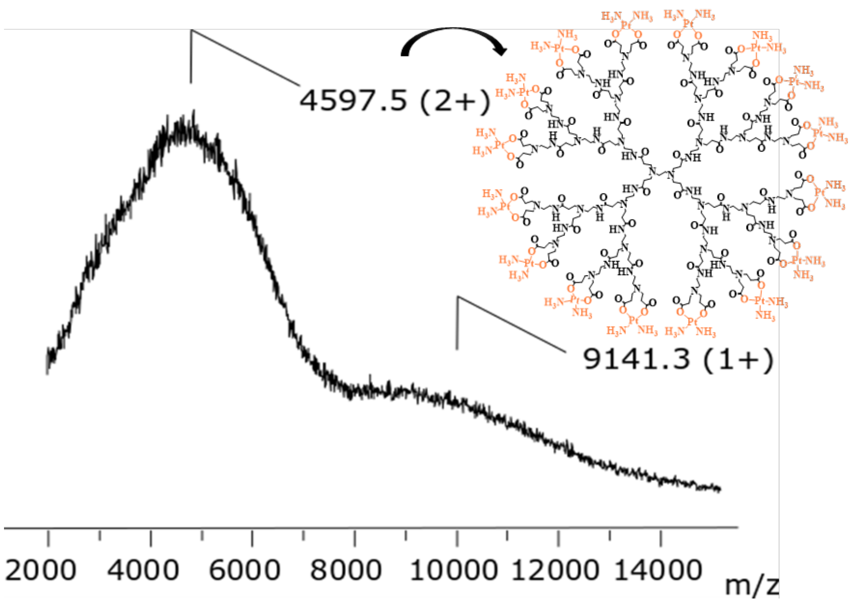



**Figure S44:** TOF-MS (MALDI) mass spectrum of bidentate G2.5COO(Pt(NH$_3$)$_2$)$_{16}$ metallodendrimer.

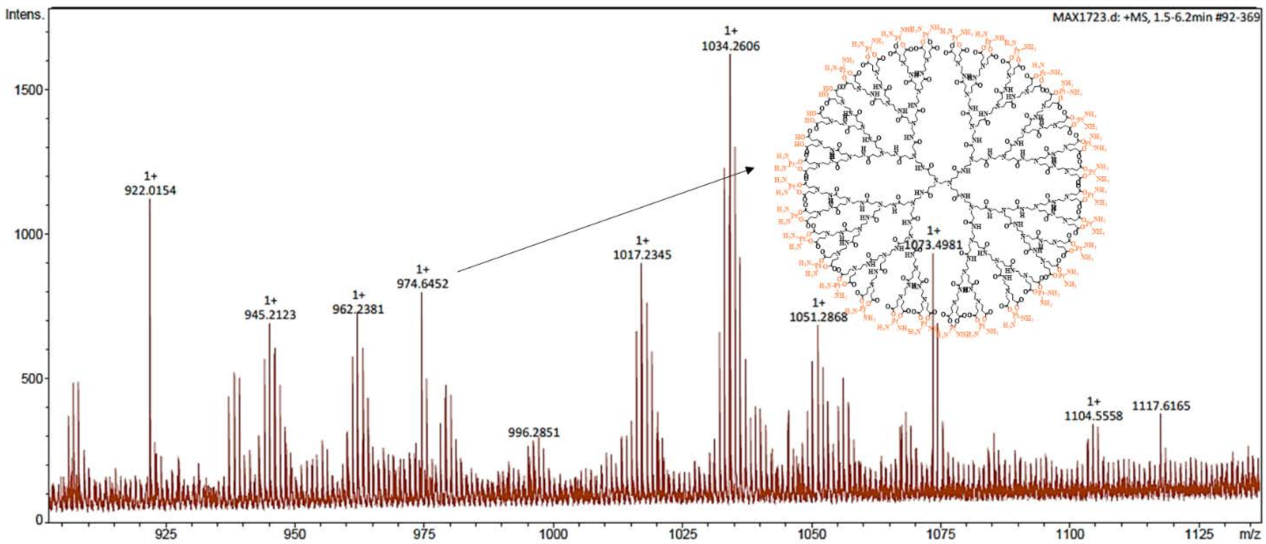



**Figure S45:** TOF-MS (ESI+) mass spectrum of bidentate G3.5COO(Pt($NH_3$)$_2$)$_{32}$ metallodendrimer.

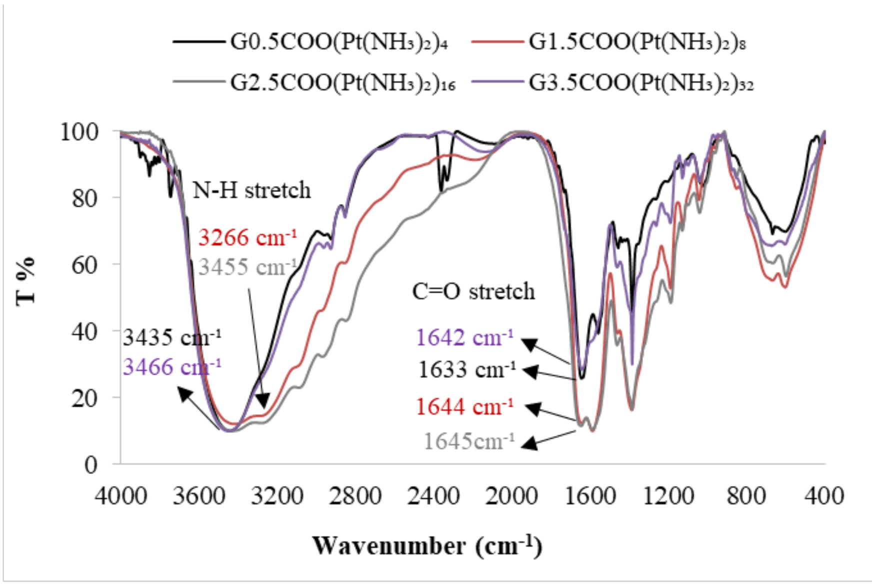
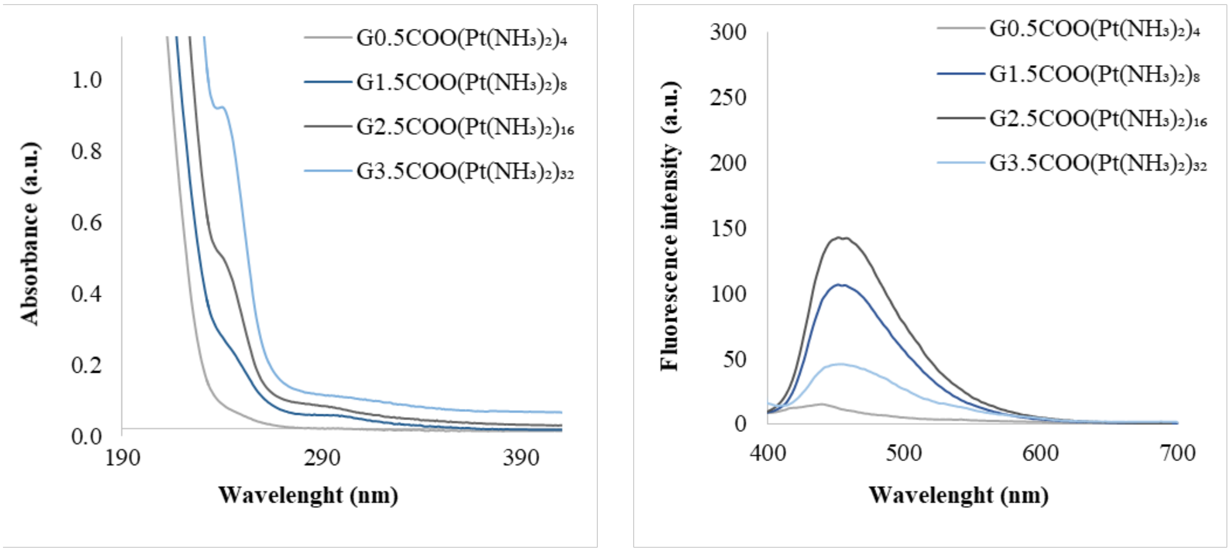



**Figure S46:** FTIR spectrum of metallodendrimers conjugated with cisplatin in bidentate form. The spectrum was performed in KBr pellet.




(a) (b)

**Figure S47:** a) Absorption spectra of cisplatin-metallodendrimers in the bidentate form recorded at a concentration of 40 µM in ultrapure water and b) Emission ($\lambda_{ex}$ = 380 nm) spectra of cisplatin-metallodendrimers in the bidentate form recorded at a concentration of 500 µM in ultrapure water.

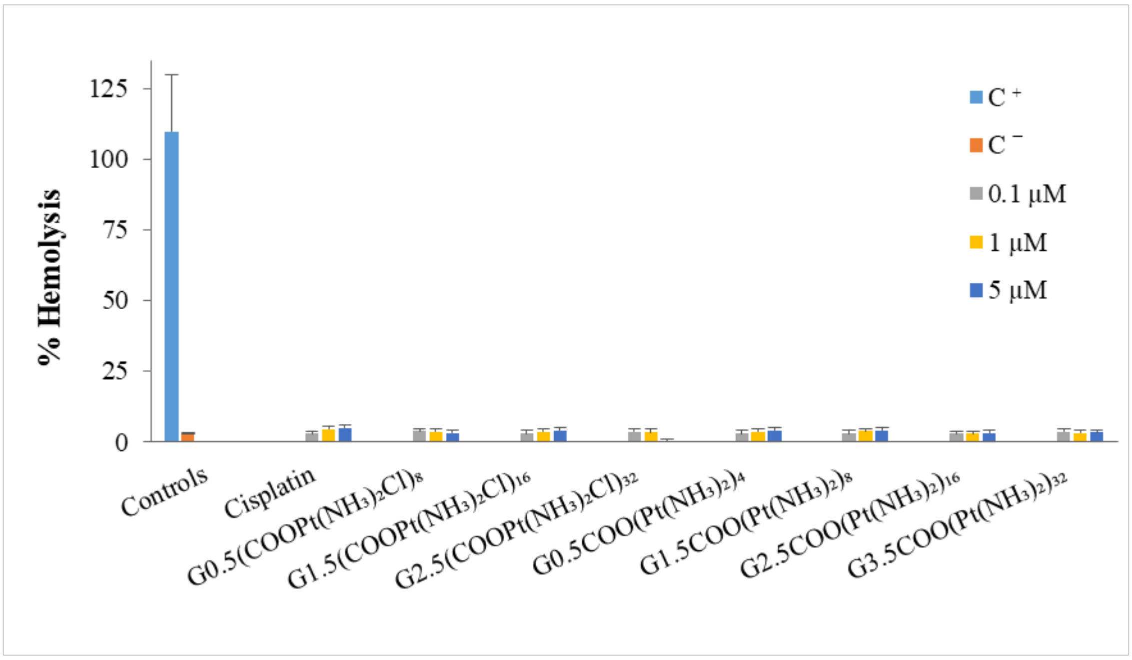



**Figure S48:** Hematoxicity of the free cisplatin and prepared cisplatin-metallodendrimers in healthy human blood. Blood was treated for 3 h with different concentrations (0.1, 1, and 5 µM) of the metallodendrimers and free cisplatin. The positive and negative control are represented by $C^+$ and $C^-$, respectively. The results are expressed as mean ± SD of at least three independent experiments performed in triplicate.

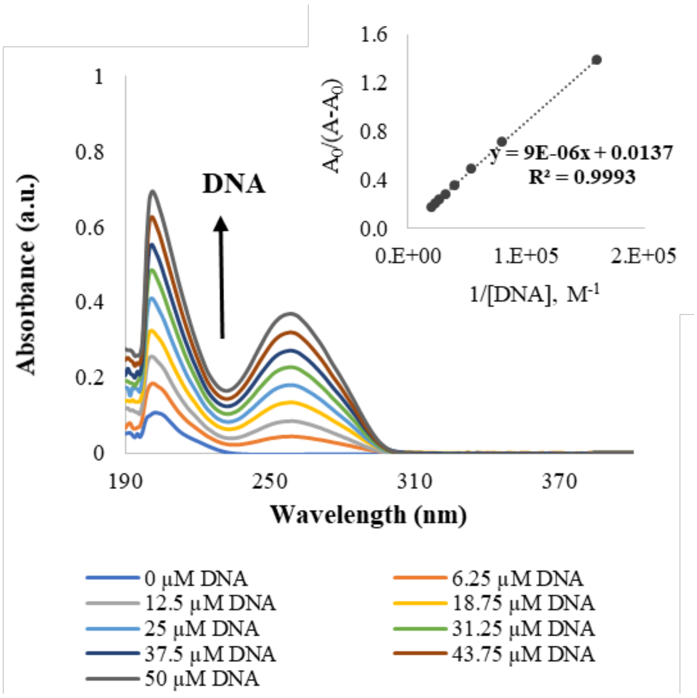



**Figure S49:** Representative UV-visible spectra of cisplatin with increasing concentration of CT-DNA (0, 6.25, 12.5, 18.75, 25, 31.25, 37.5, 43.75 and 50 µM) in 5 mM Tris-HCl/50 mM NaCl at pH 7.4. The inset corresponds to the plot of $A_0/(A-A_0)$ versus 1/[DNA], which is used to determine the binding constant. The arrow indicates the direction of increasing the concentration of DNA.