

# Stepwise Optimization of Tumor-Targeted Dual-Action Platinum(IV)-Gemcitabine Prodrugs

## Supporting information

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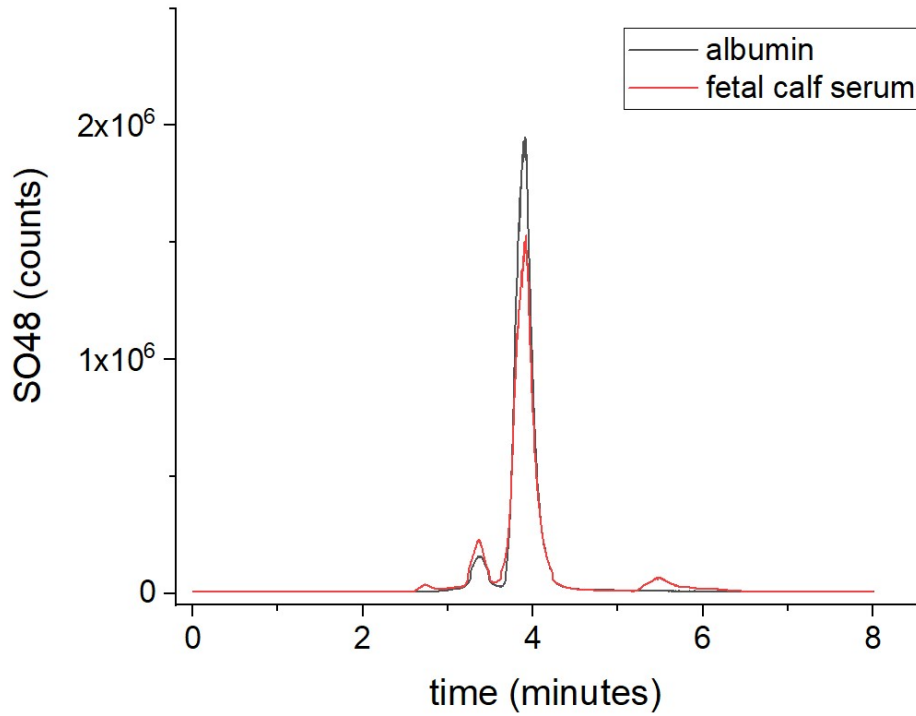
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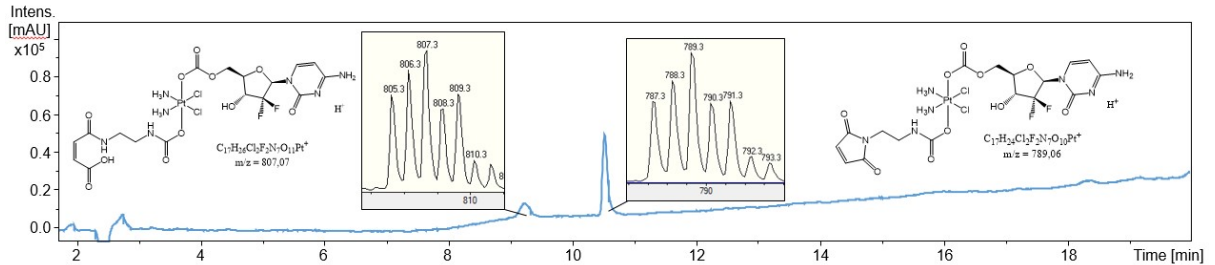
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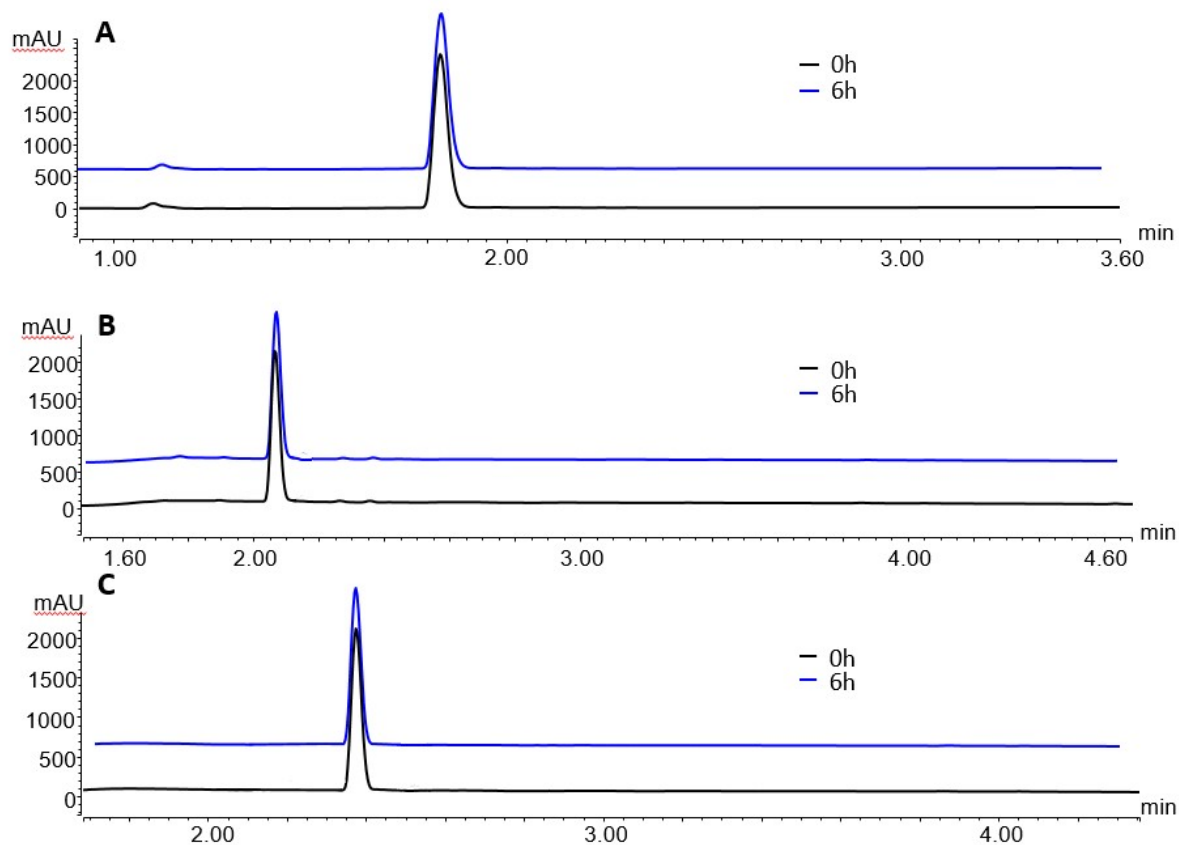
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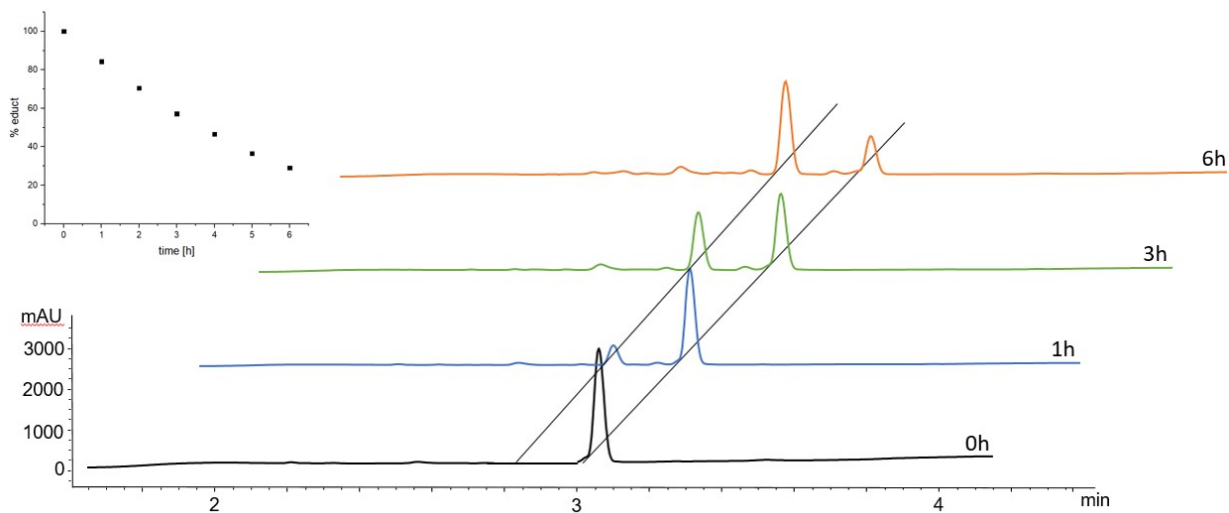
**Figure S1:** Sulfur traces of fetal calf serum (+150 mM phosphate buffer, pH 7.4) as well as pure albumin in phosphate buffer (50 mM, pH7.4), both measured by SEC-ICP-MS. The small peak at ~3.3 min corresponds to the albumin dimer.



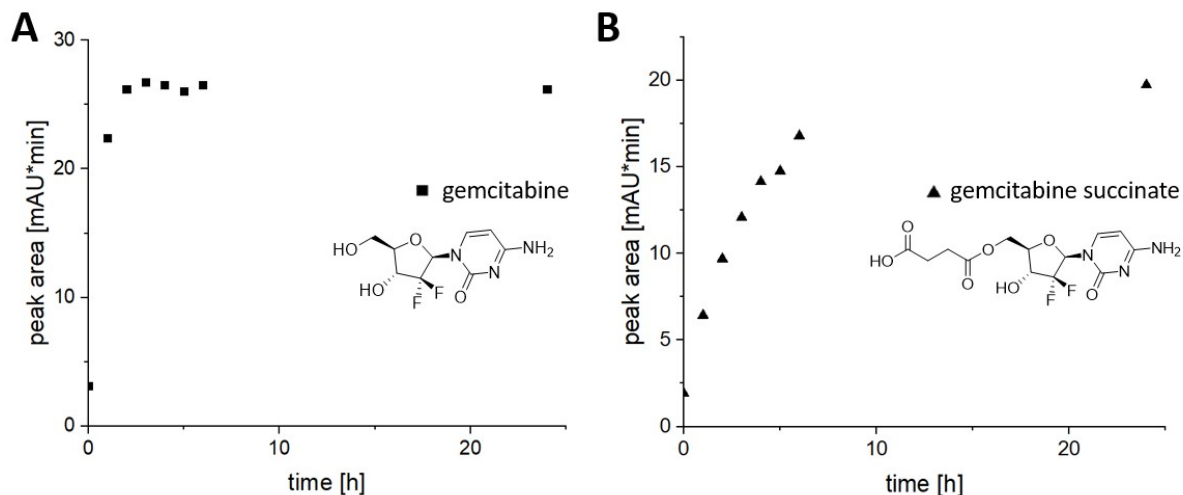
**Figure S2:** Peak assignment via HPLC-MS of the stability experiment for **CisPt-GemCarb-C<sub>2</sub>Mal** depicted in Figure 3.



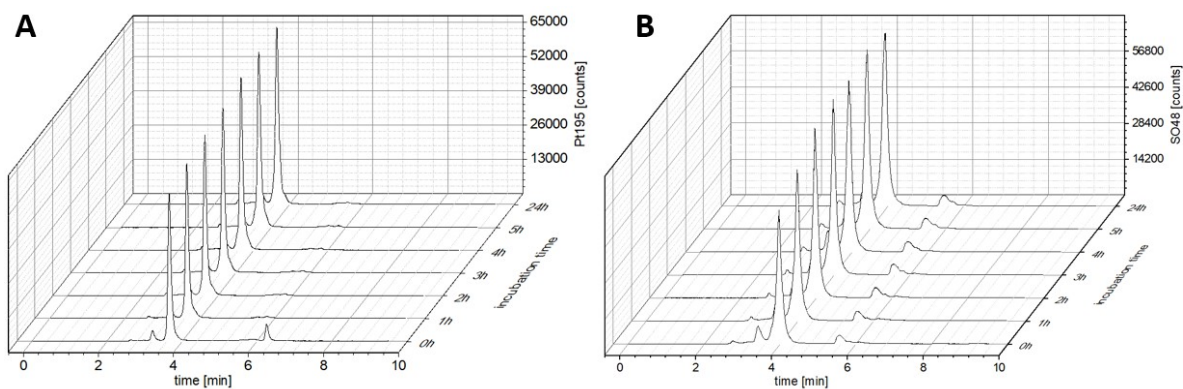
**Figure S3.** UV/Vis-traces (220 nm) of stability measurements of 1 mM **CisPt-GemCarb-OAc (A)**, **CisPt-GemSucc-OAc (B)** and **CarboPt-GemSucc-OAc (C)** in phosphate buffer (150 mM, pH 7.4) at 37 °C, measured with UHPLC.



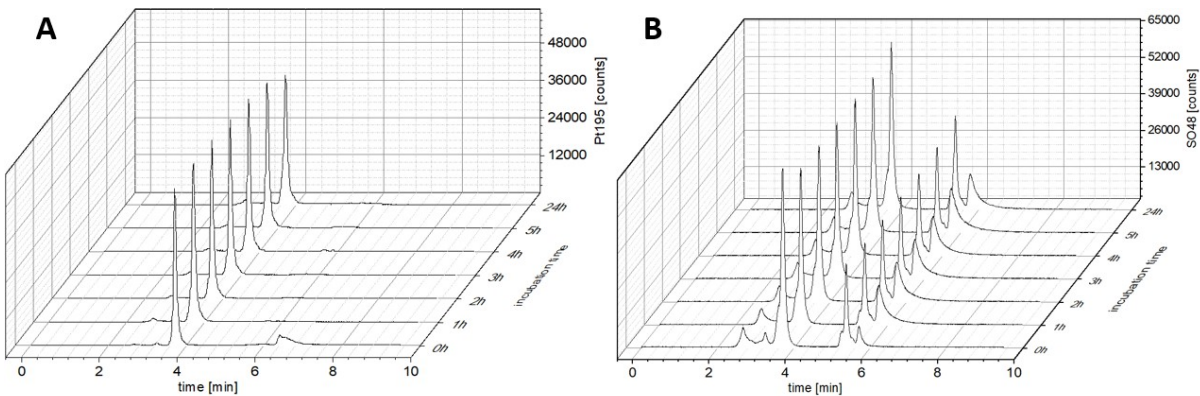
**Figure S4.** Maleimide hydrolysis of 1 mM **CisPt-GemSucc-C<sub>5</sub>Mal** in phosphate buffer (150 mM, pH 7.4) at 37 °C, monitored with UHPLC (220 nm).



**Figure S5:** A) Gemcitabine release of the reduction kinetics of **CisPt-GemCarb-OAc** and (B) gemcitabine succinate release of the reduction kinetics of **CisPt-GemSucc-OAc**. The data were measured with UHPLC at 1 mM drug concentration in phosphate buffer (150 mM, pH 7.4) at 20 °C with 10 eq of ascorbic acid over 24 h.



**Figure S6:** <sup>195</sup>Platinum (A) and <sup>48</sup>sulfur (B) traces of 100 μM **CisPt-GemSucc-C<sub>5</sub>Mal** incubated in FCS (buffered with 150 mM phosphate buffer, pH 7.4) at 37 °C, measured with ICP-MS.



**Figure S7:** <sup>195</sup>Platinum (A) and <sup>48</sup>sulfur (B) traces of 100 μM **CarboPt-GemSucc-C<sub>5</sub>Mal** incubated in FCS (buffered with 150 mM phosphate buffer, pH 7.4) at 37 °C, measured with ICP-MS.

HPLC column:	Acquity UPLC BEH 200Å 1.7 µm, 4.6x150 mm
Eluent:	50 mM CH <sub>3</sub> COONH <sub>4</sub> , pH = 6.8
Flow rate:	400 µL/min
Injection volume:	0.5 µL
Column temperature:	37 °C
Autosampler temperature:	37 °C

**Table S1:** SEC-HPLC parameters for SEC-ICP-MS measurements.

Nebulizer:	Quartz
Spray chamber:	Scott type
Nebulizer gas flow:	1.08 L/min
Aux. gas flow:	0.9 L/min
Plasma gas flow:	15 L/min
Reaction gas (oxygen):	30 %
ICP RF power:	1550 W
m/z measured:	195, 48

**Table S2:** ICP-MS parameters for ICP-MS measurements.

# CisPt-GemCarb-OAc

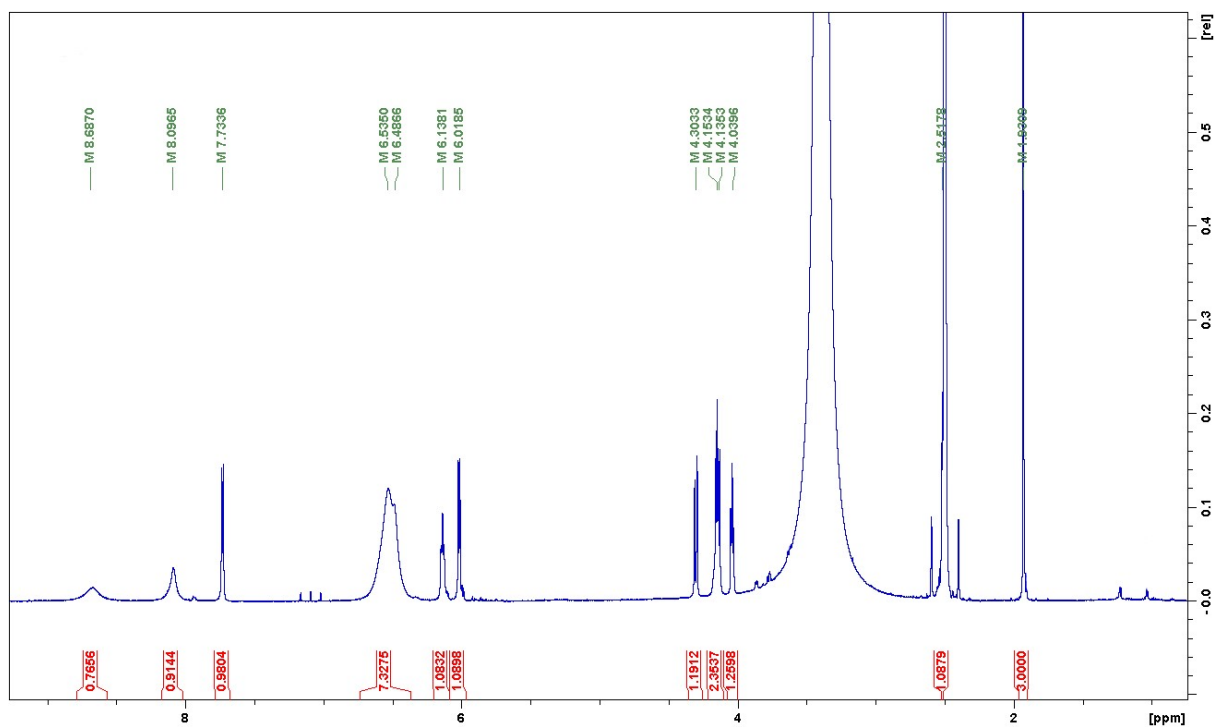


Figure S8: <sup>1</sup>H-NMR of CisPt-GemCarb-OAc.

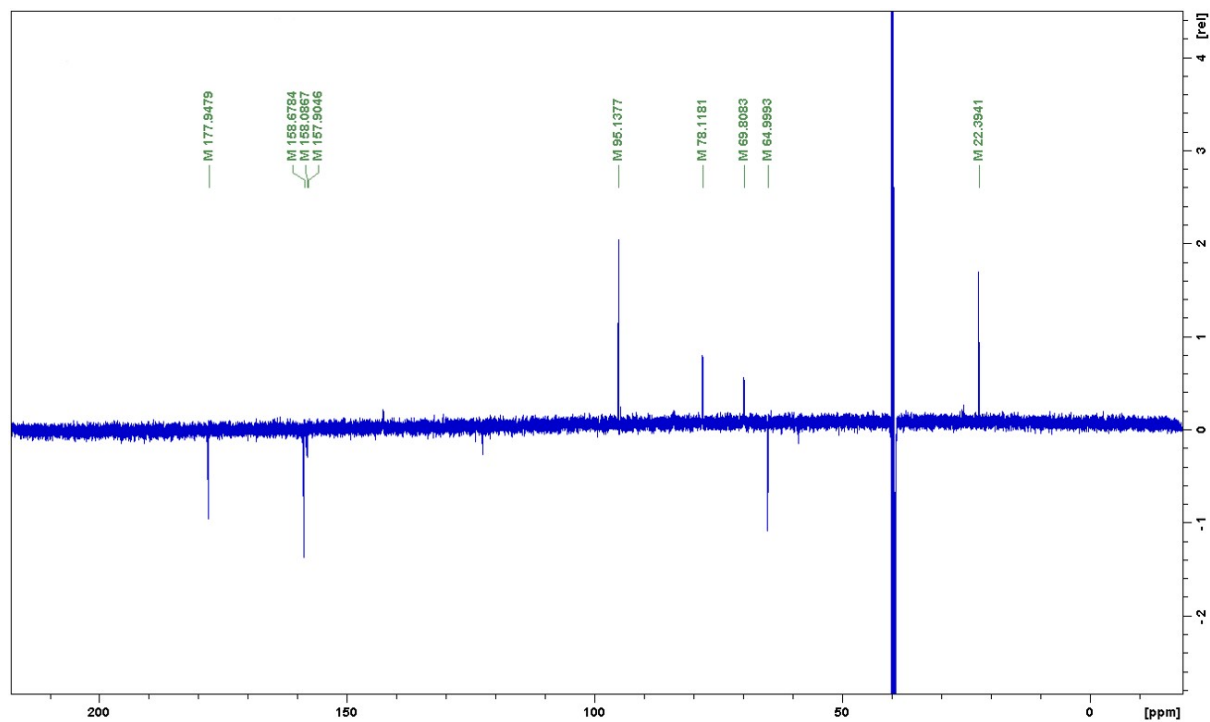
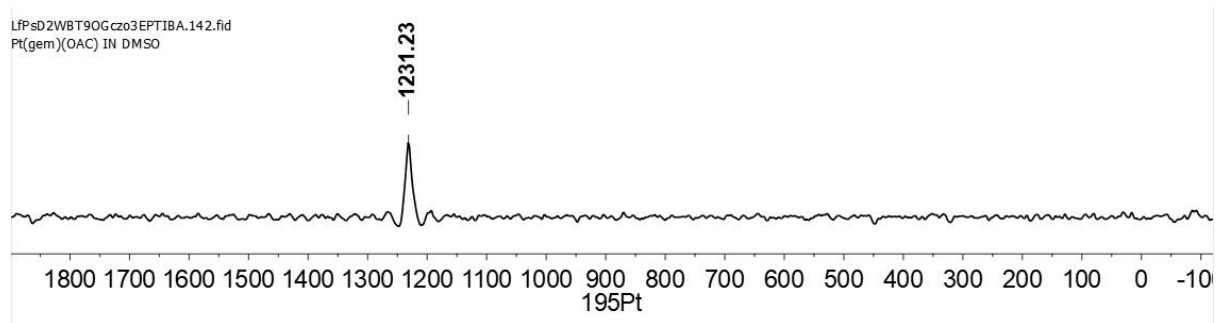
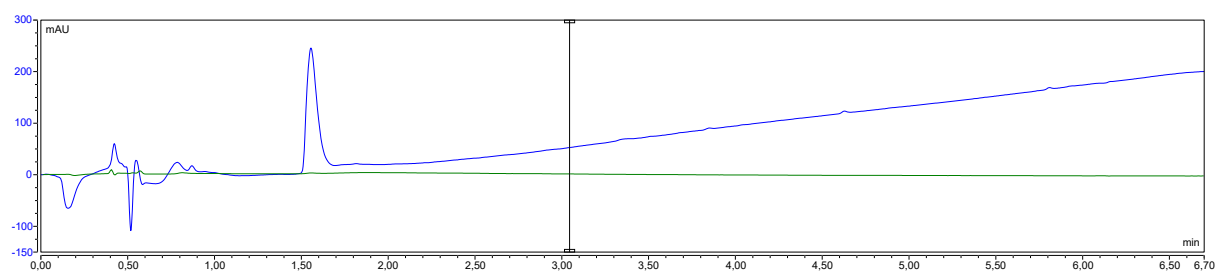


Figure S9: <sup>13</sup>C-NMR of CisPt-GemCarb-OAc.



**Figure S10:**  $^{195}\text{Pt}$ -NMR of CisPt-GemCarb-OAc.



**Figure S11:** RP-HPLC chromatogram of CisPt-GemCarb-OAc.

### CisPt-GemCarb-C<sub>2</sub>Mal

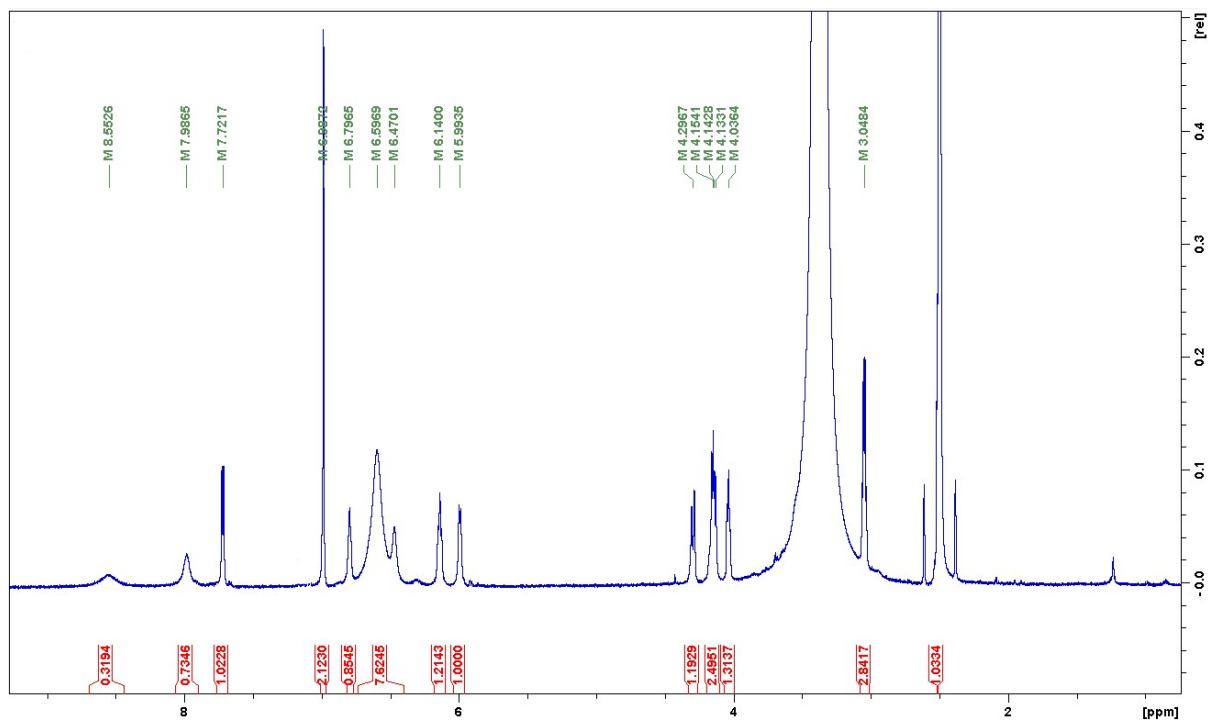


Figure S12: <sup>1</sup>H-NMR of CisPt-GemCarb-C<sub>2</sub>Mal.

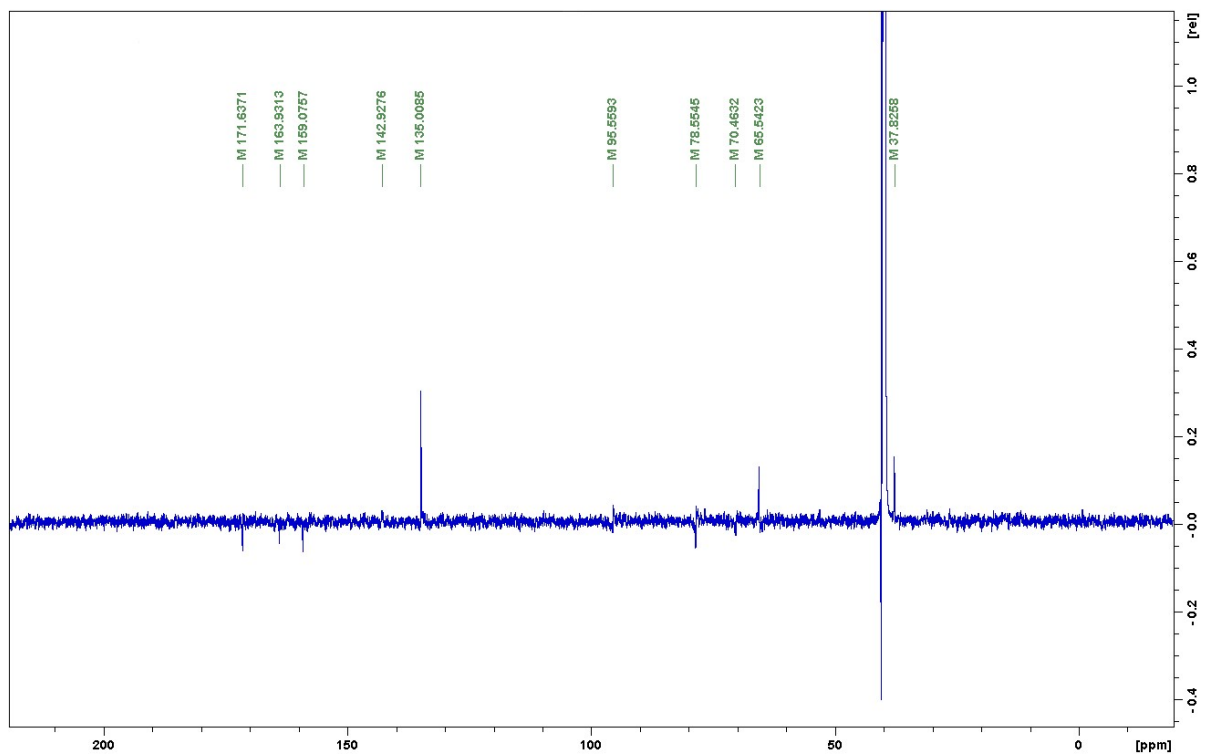


Figure S13: <sup>13</sup>C-NMR of CisPt-GemCarb-C<sub>2</sub>Mal.



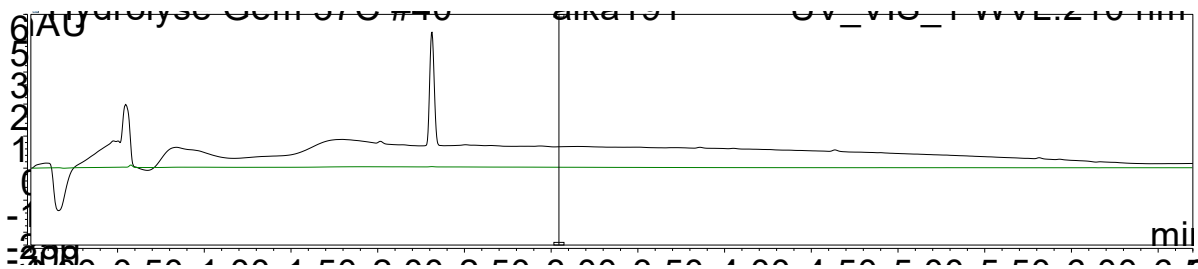


Figure S14: RP-HPLC chromatogram of CisPt-GemCarb-C<sub>2</sub>Mal.

CisPt-GemCarb-C<sub>5</sub>Mal

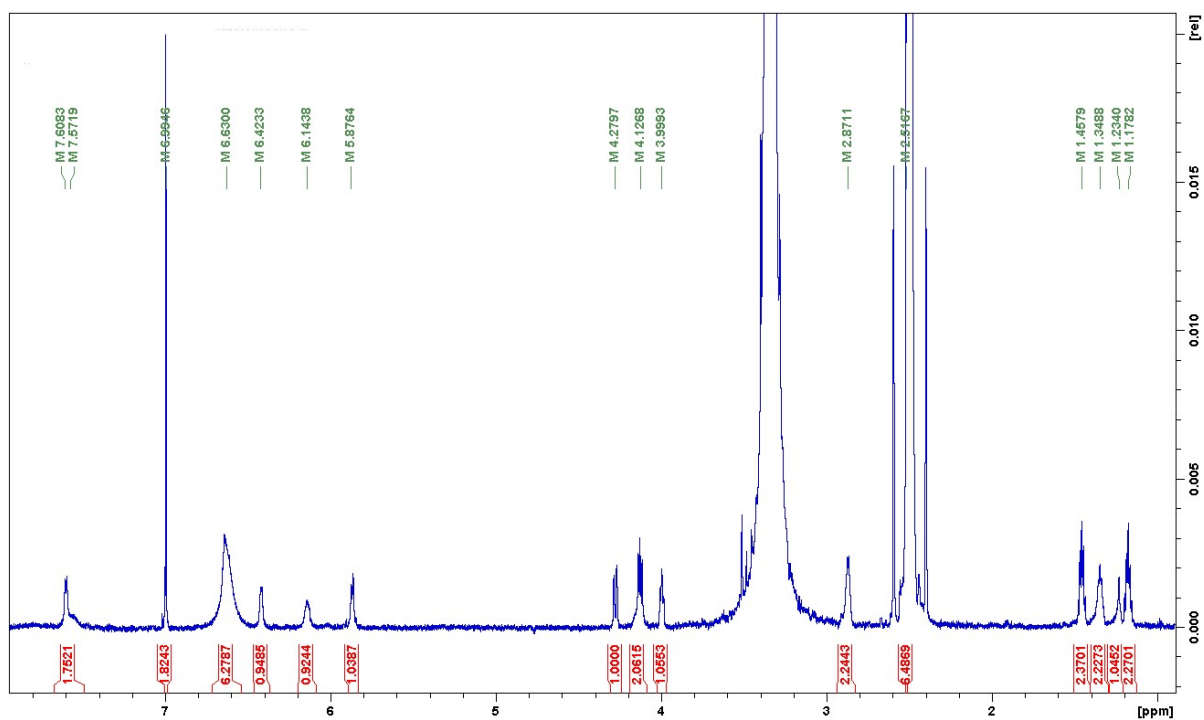


Figure S15: <sup>1</sup>H-NMR of CisPt-GemCarb-C<sub>5</sub>Mal.

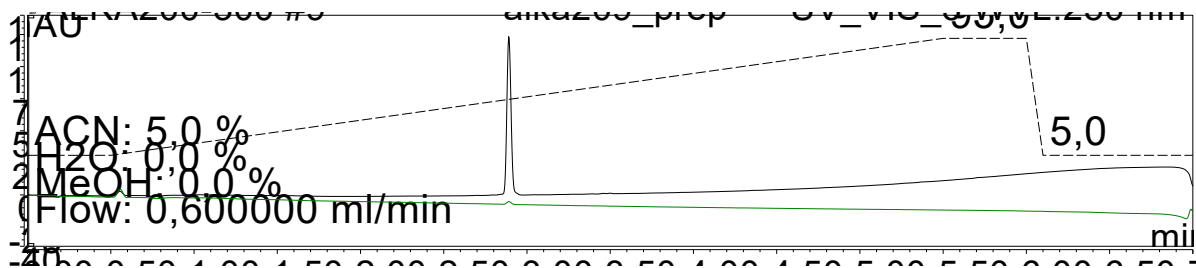


Figure S16: RP-HPLC chromatogram of CisPt-GemCarb-C<sub>5</sub>Mal.

### CisPt-GemSucc-OAc

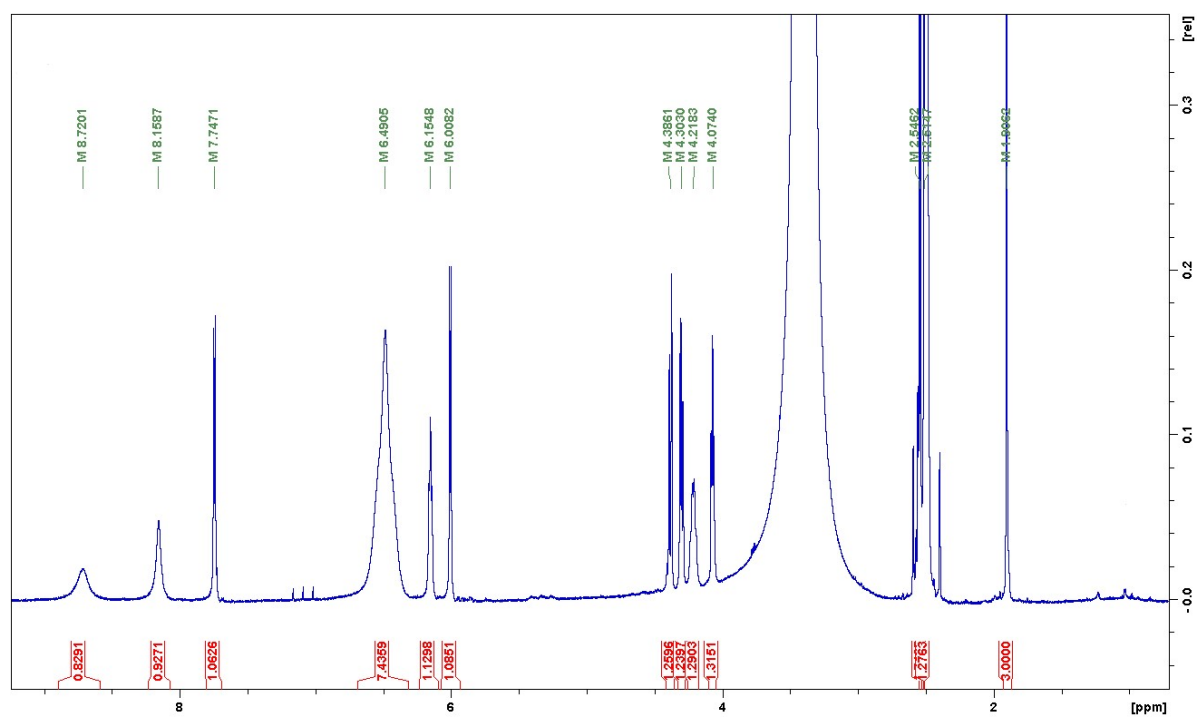


Figure S17: <sup>1</sup>H-NMR of CisPt-GemSucc-OAc.

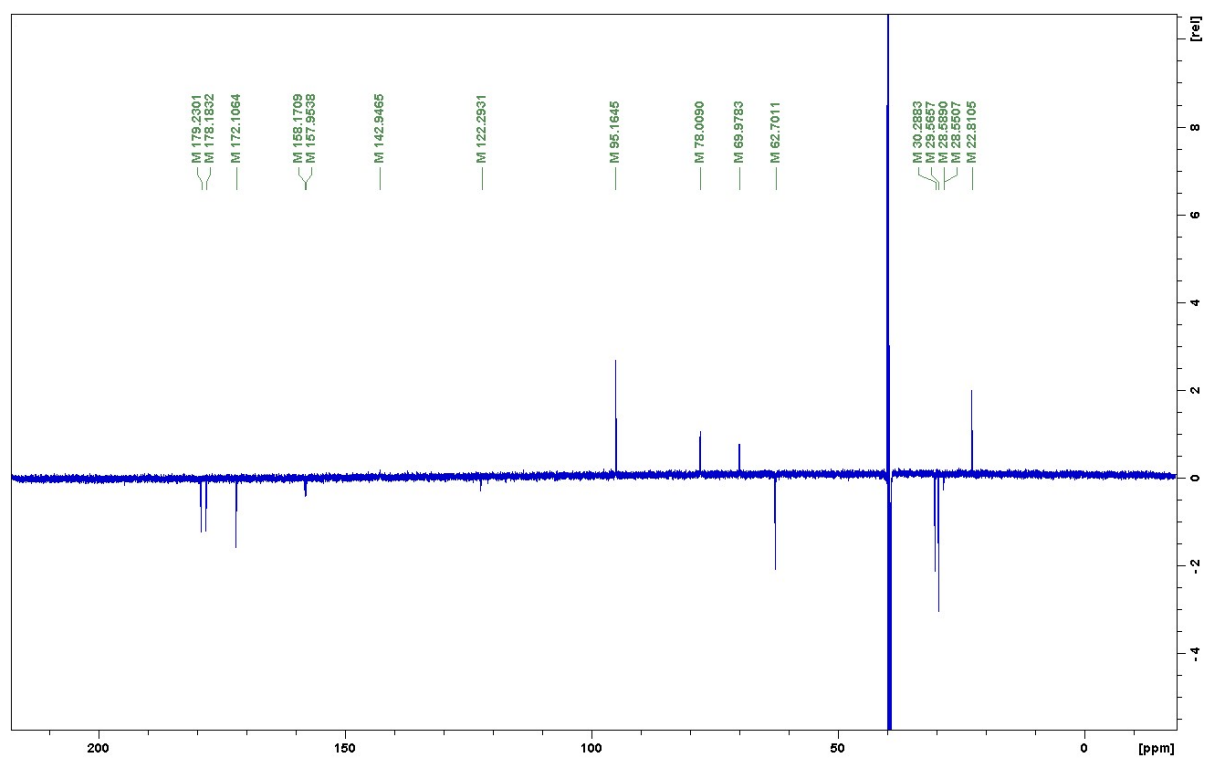
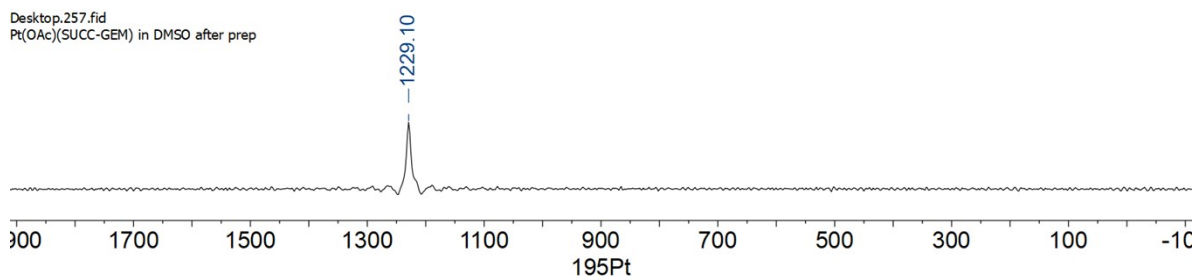
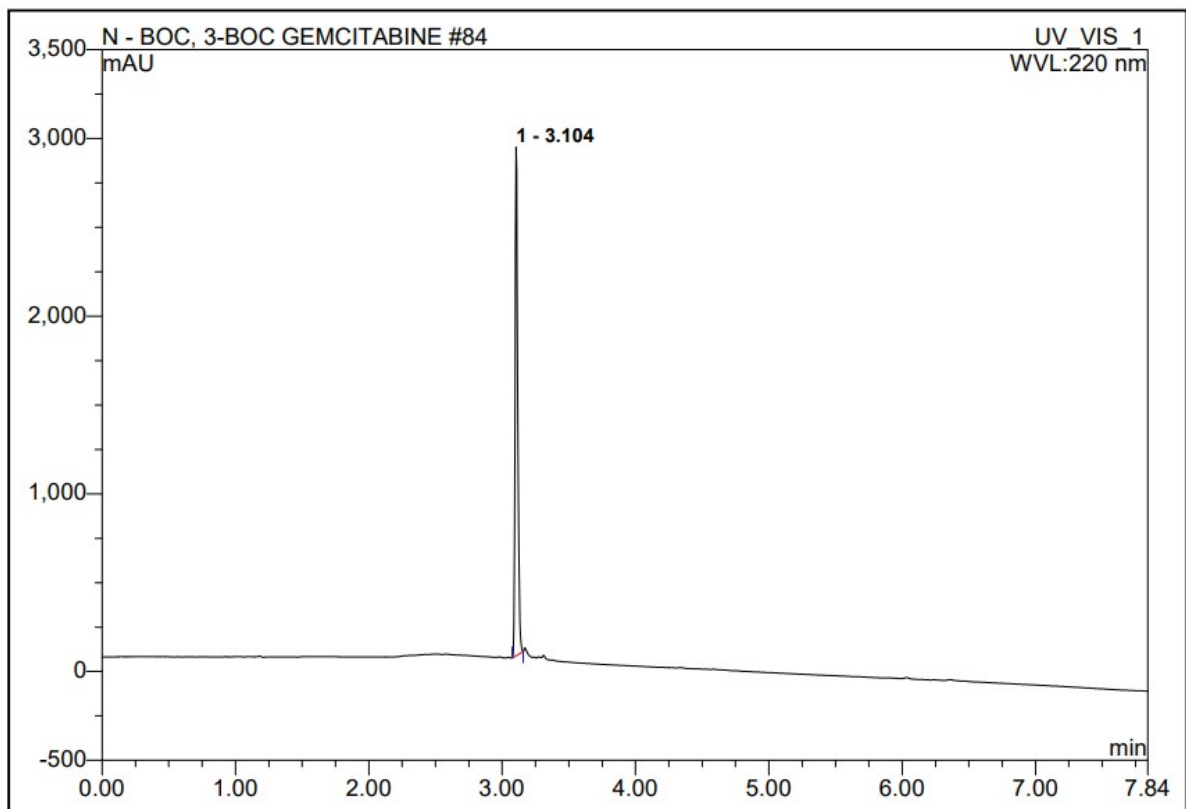


Figure S18: <sup>13</sup>C-NMR of CisPt-GemSucc-OAc.



**Figure S19:**  $^{195}\text{Pt}$ -NMR of CisPt-GemSucc-OAc.



**Figure S20:** RP-HPLC chromatogram of CisPt-GemSucc-OAc.

### CisPt-GemSucc-C<sub>5</sub>Mal

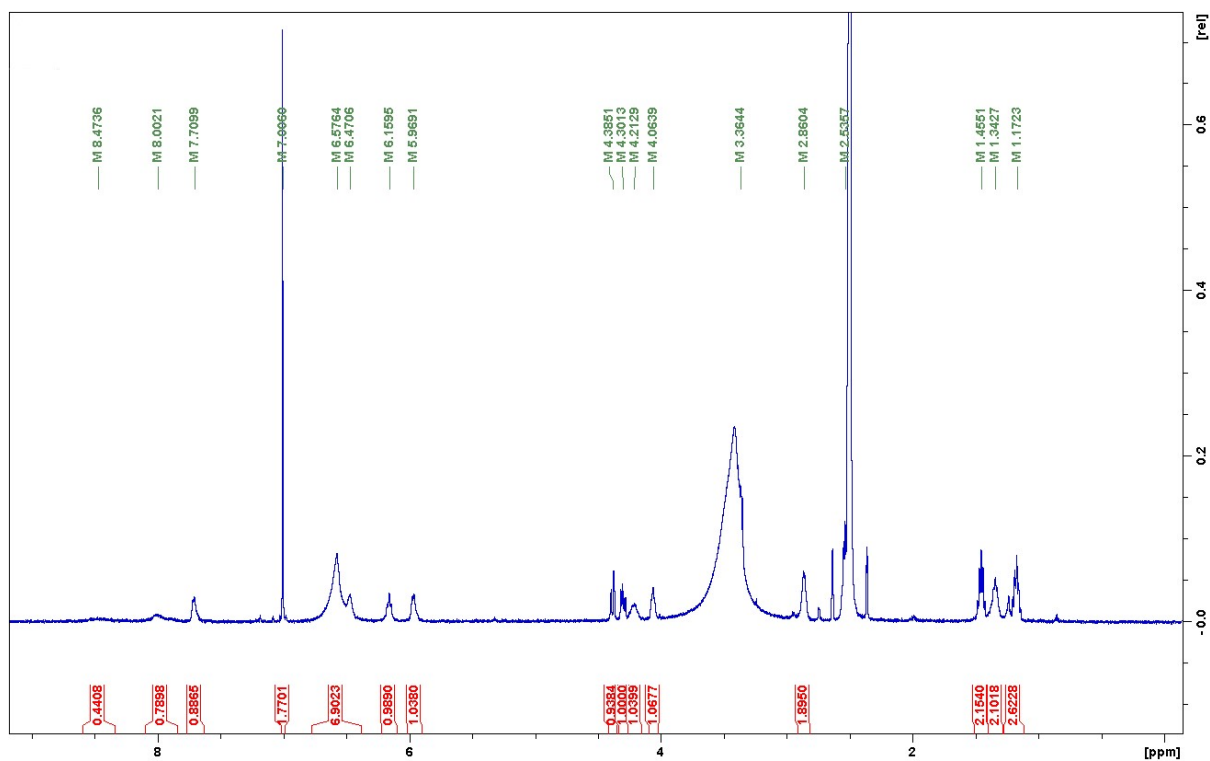


Figure S21: <sup>1</sup>H-NMR of CisPt-GemSucc-C<sub>5</sub>Mal.

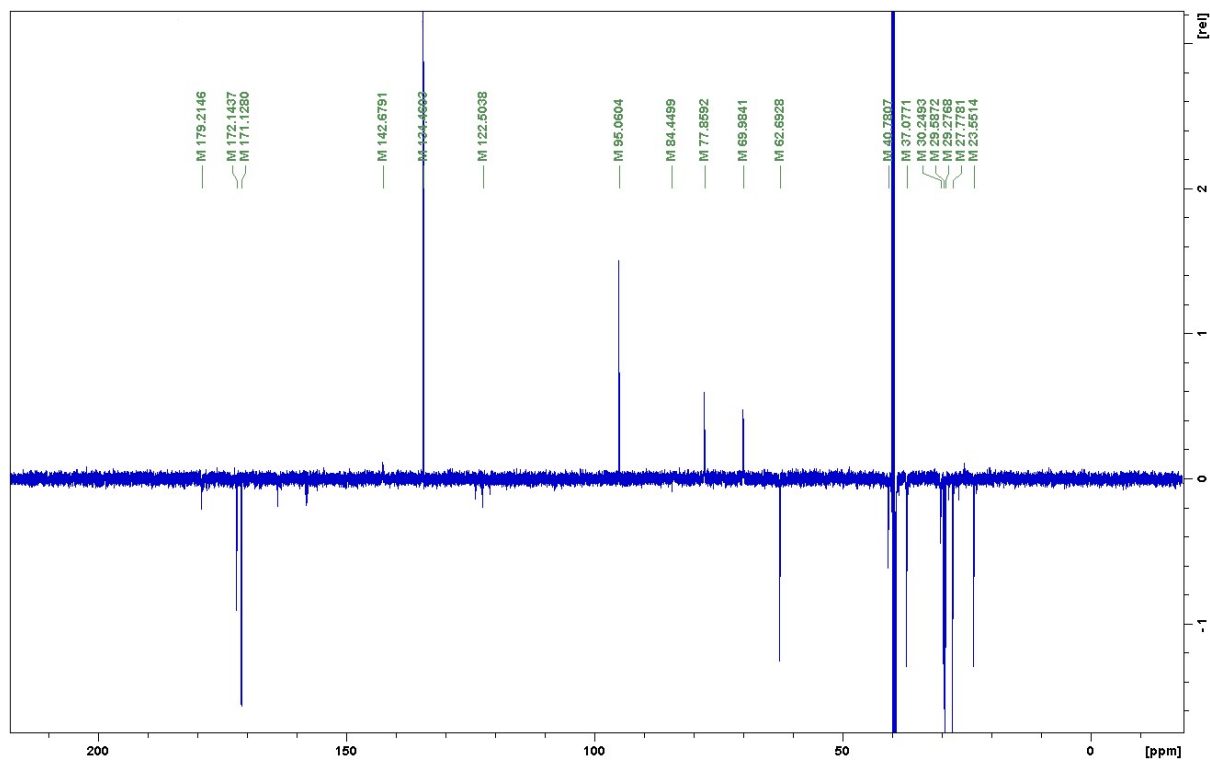
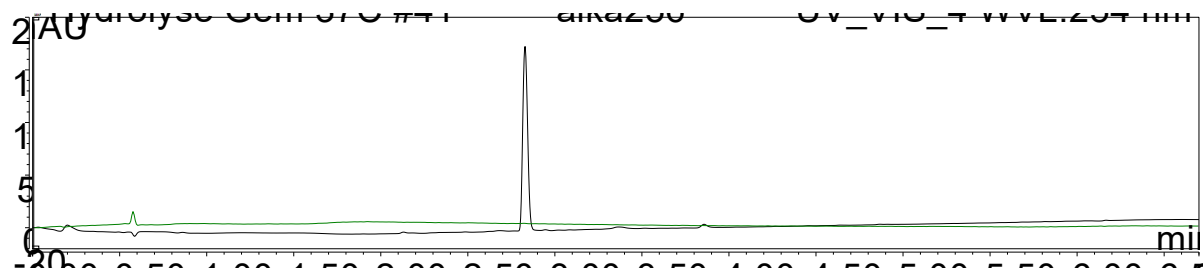


Figure S22: <sup>13</sup>C-NMR of CisPt-GemSucc-C<sub>5</sub>Mal.



**Figure S23:** RP-HPLC chromatogram of CisPt-GemSucc-C<sub>5</sub>Mal.

# CarboPt-GemSucc-OAc

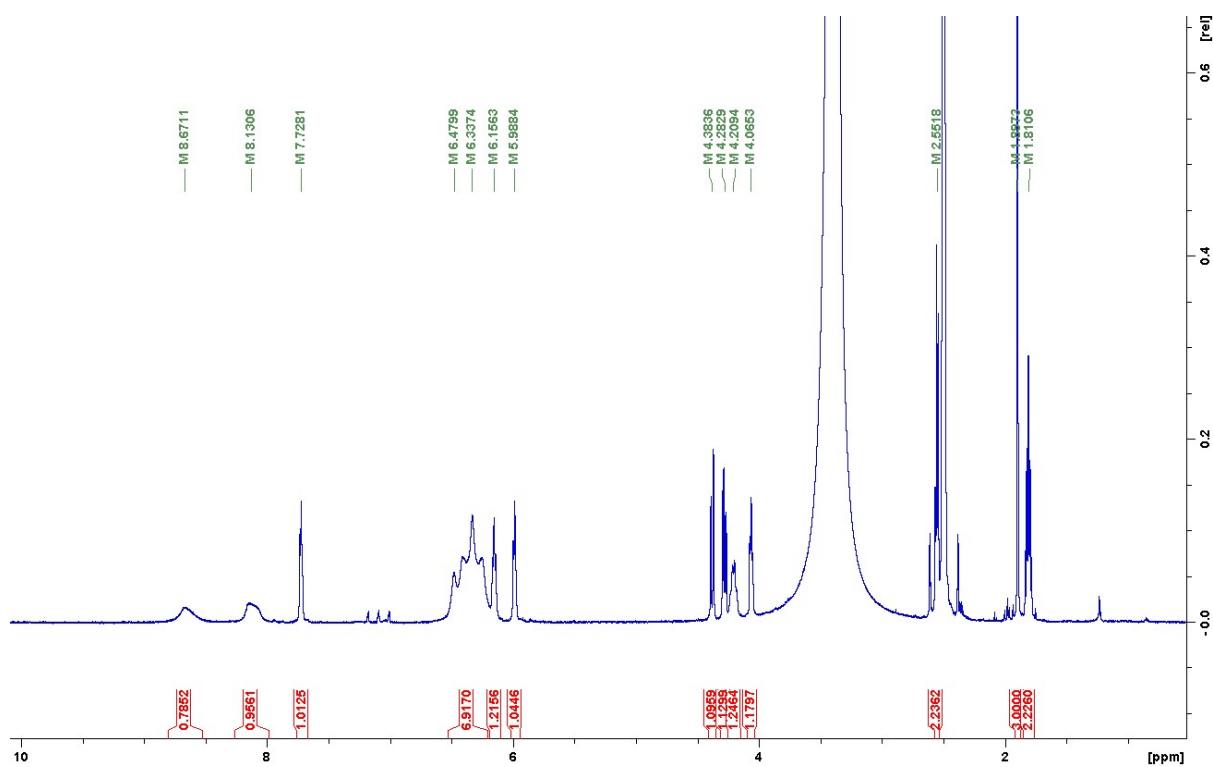


Figure S24: <sup>1</sup>H-NMR of CarboPt-GemSucc-OAc.

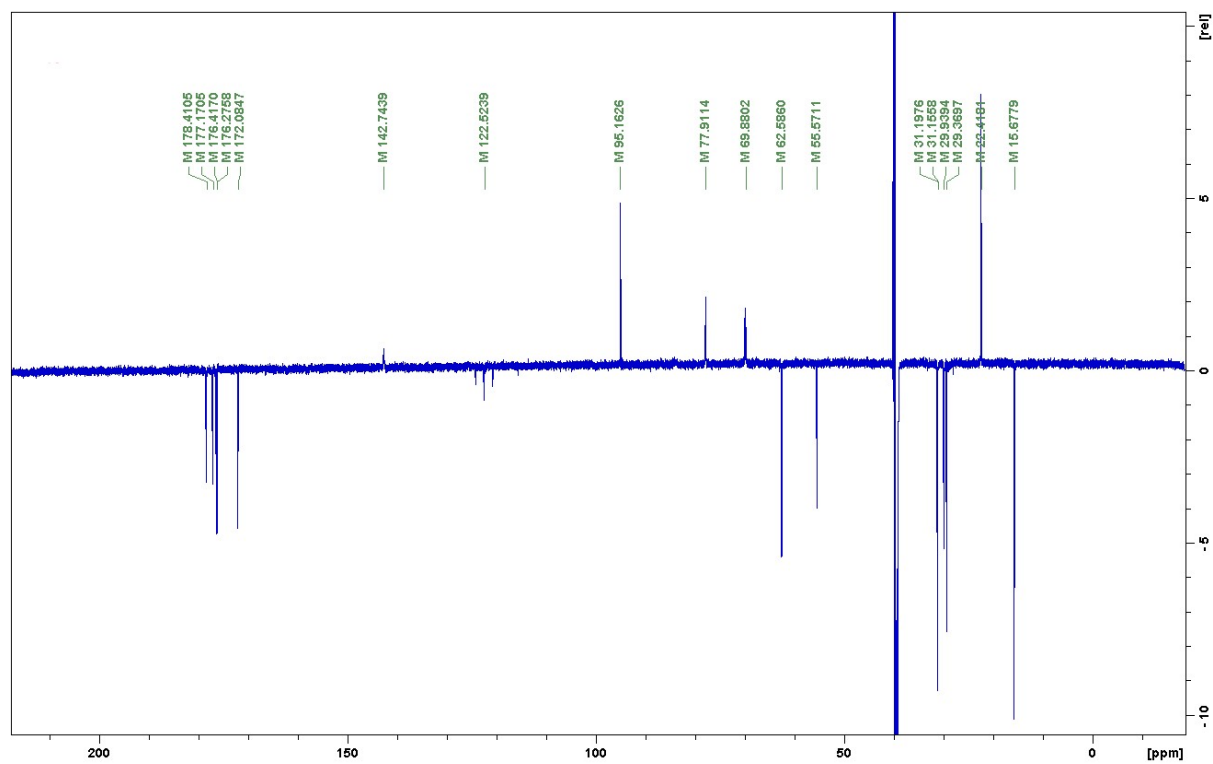


Figure S25: <sup>13</sup>C-NMR of CarboPt-GemSucc-OAc.

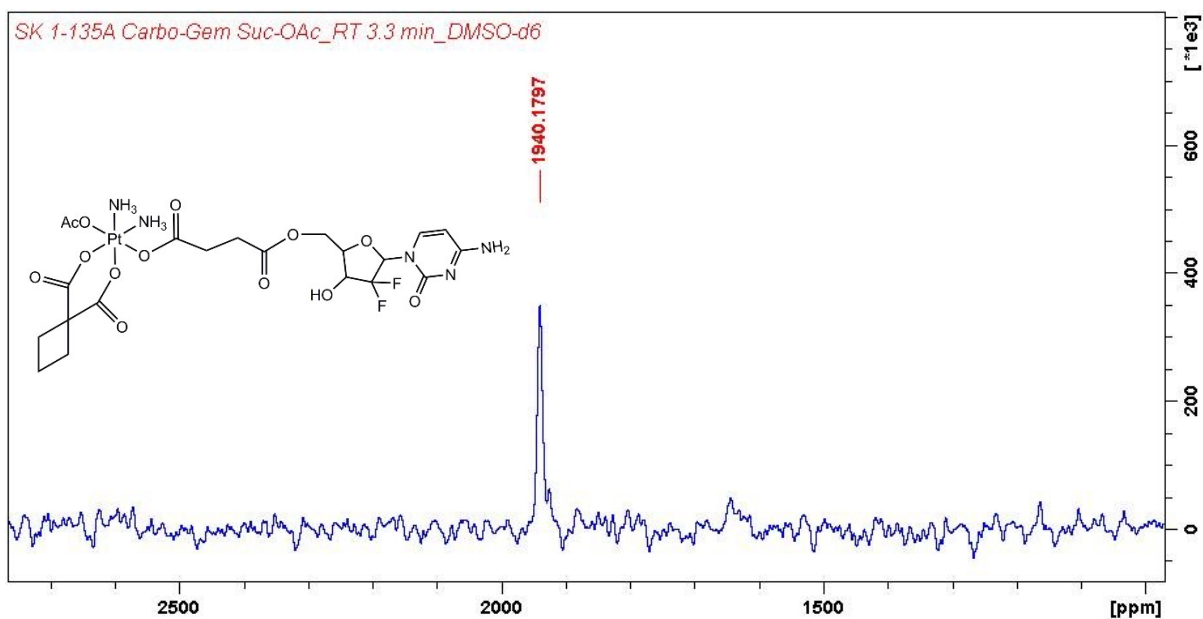
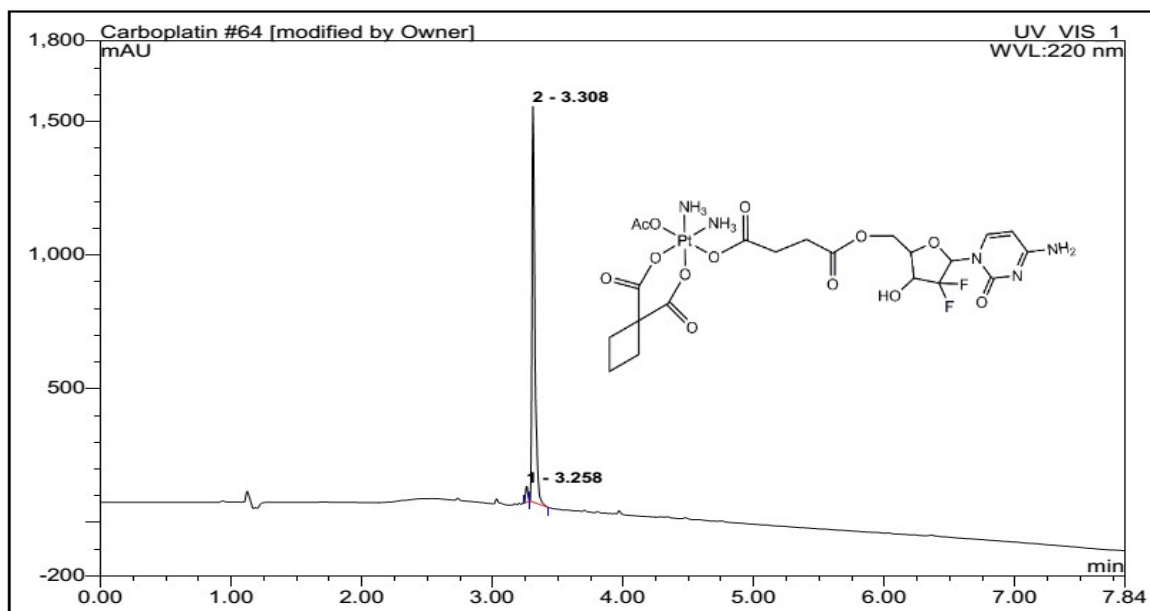


Figure S26:  $^{195}\text{Pt}$ -NMR of CarboPt-GemSucc-OAc.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.26	n.a.	59.692	1.132	2.89	n.a.	BMB*
2	3.31	n.a.	1480.308	38.094	97.11	n.a.	bMB*
<b>Total:</b>			1540.000	39.226	100.00	0.000	

Figure S27: RP-HPLC chromatogram of CarboPt-GemSucc-OAc.



### CarboPt-GemSucc-C<sub>5</sub>Mal

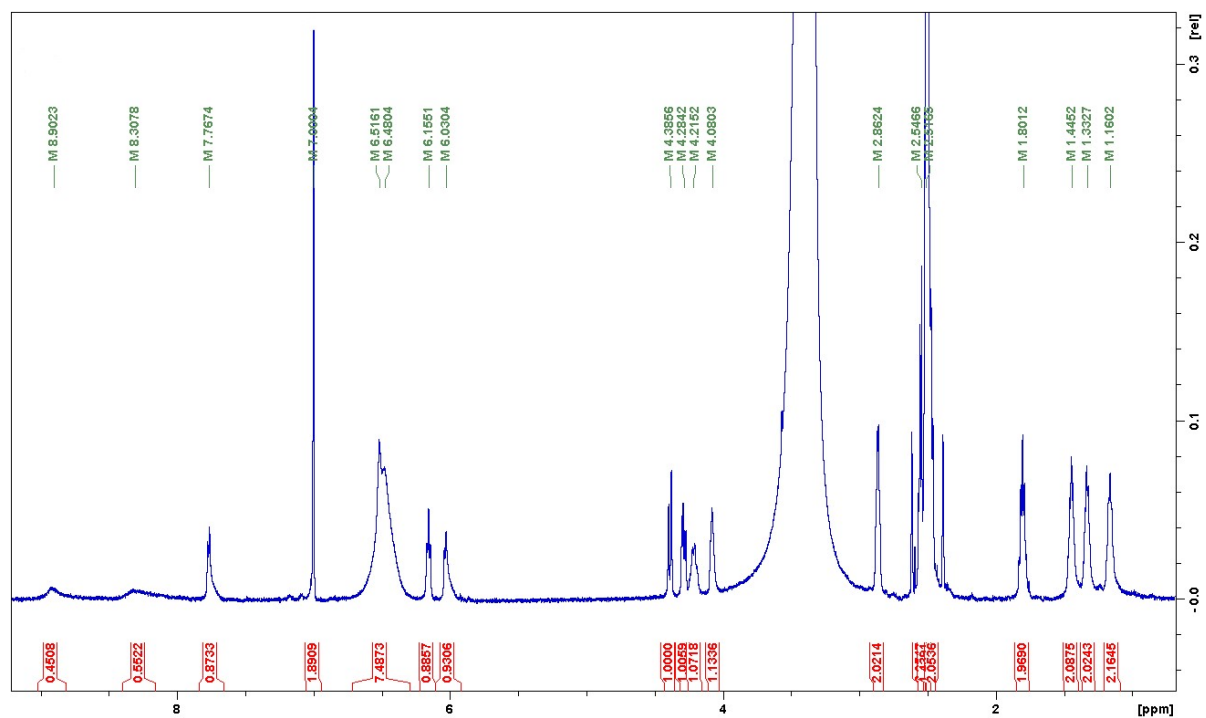


Figure S28: <sup>1</sup>H-NMR of CarboPt-GemSucc-C<sub>5</sub>Mal.

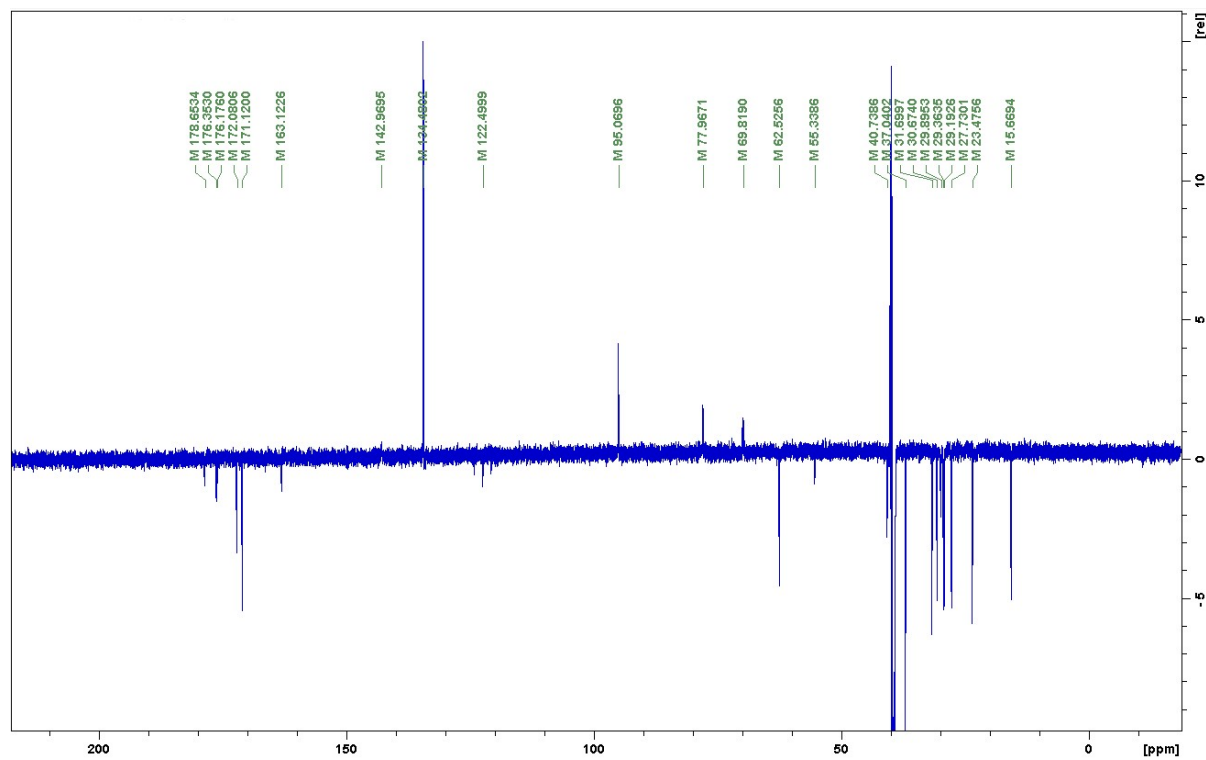
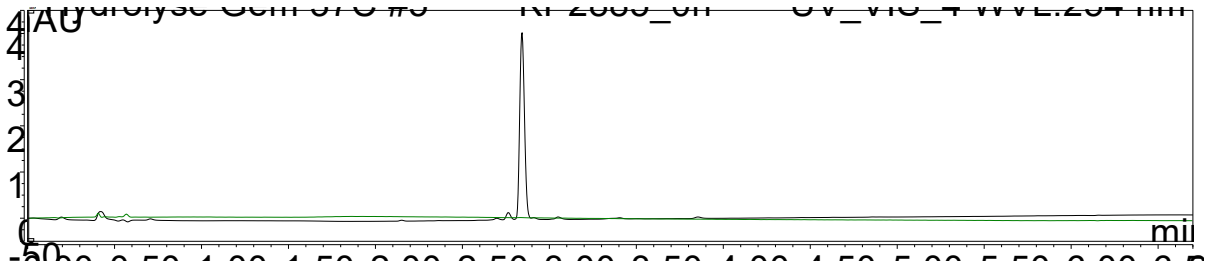


Figure S29: <sup>13</sup>C-NMR of CarboPt-GemSucc-C<sub>5</sub>Mal.



**Figure S30:** RP-HPLC chromatogram of CarboPt-GemSucc-C<sub>5</sub>Mal.