

**Electronic Supplementary Information for:**

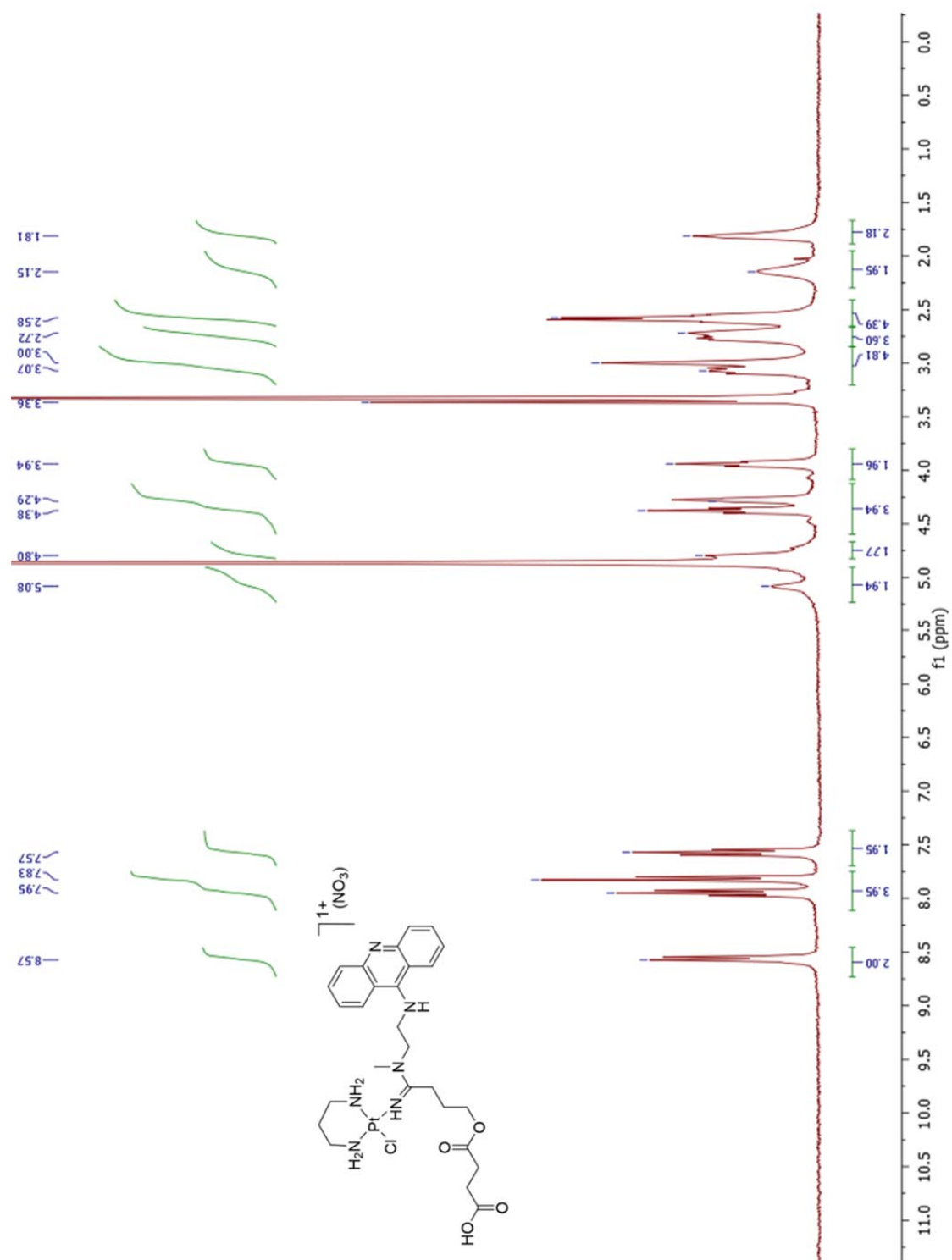
**Linker design for the modular assembly of multifunctional and targeted platinum(II)-containing anticancer agents**

Song Ding and Ulrich Bierbach

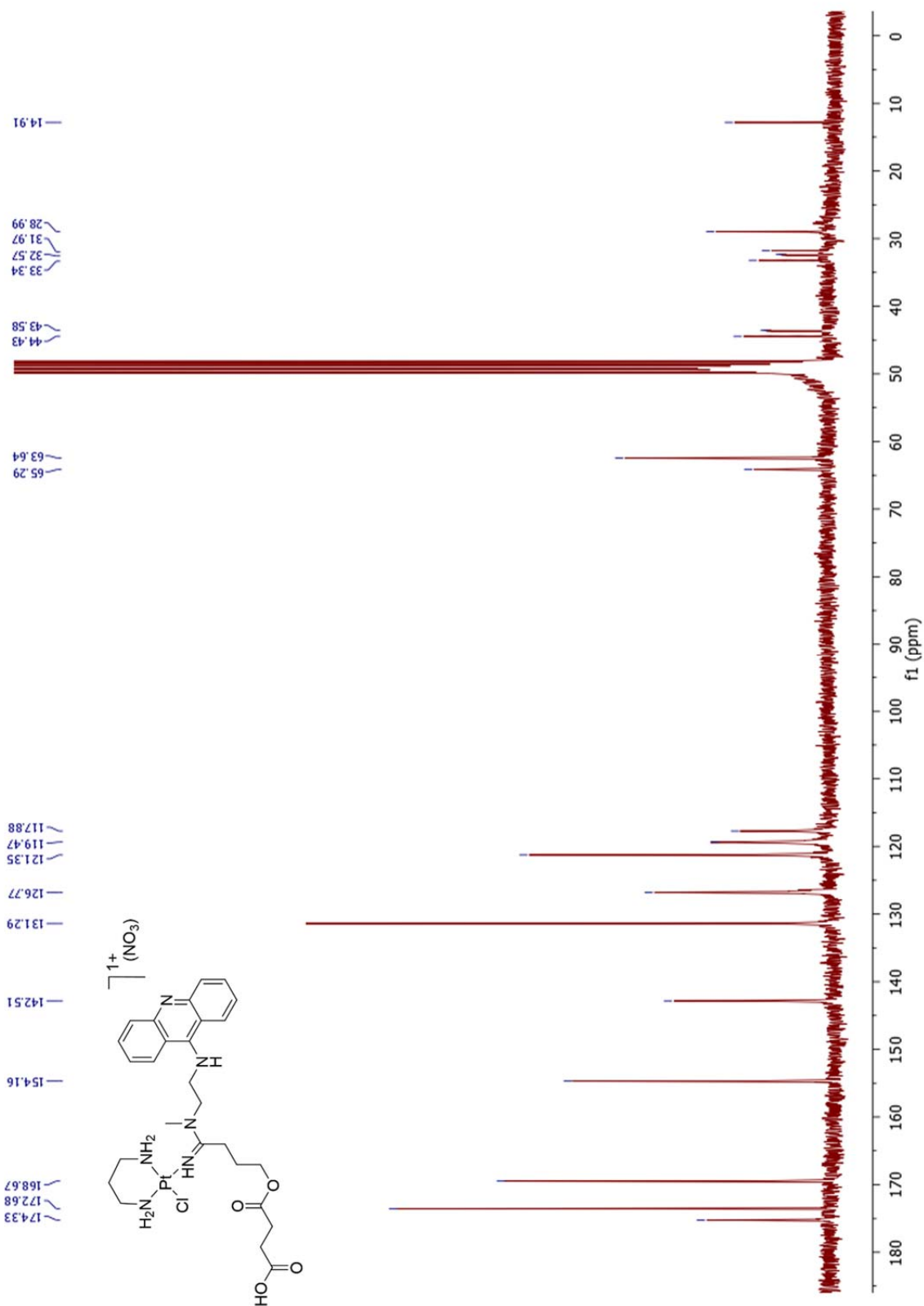
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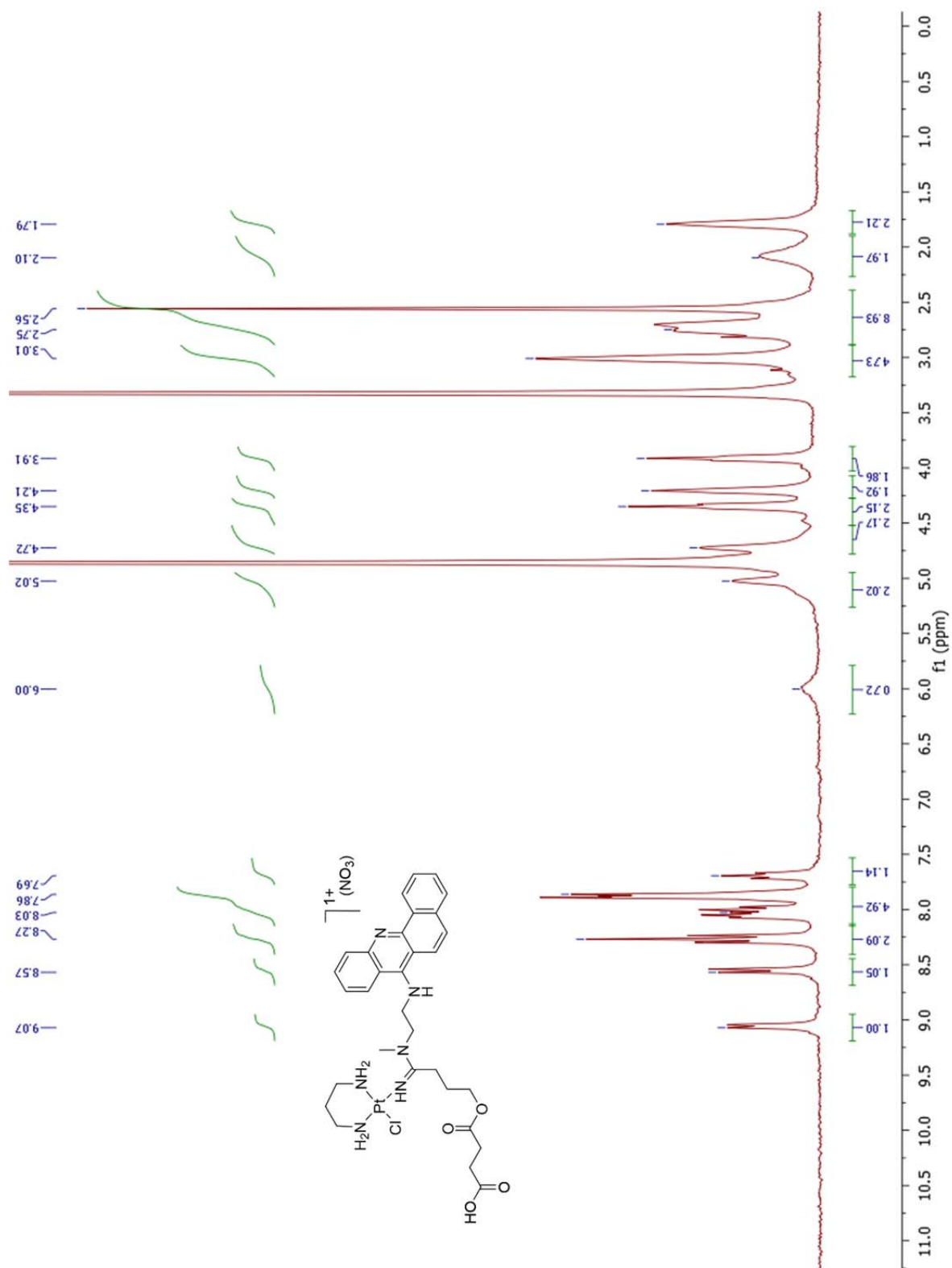
## S1. NMR SPECTROSCOPY



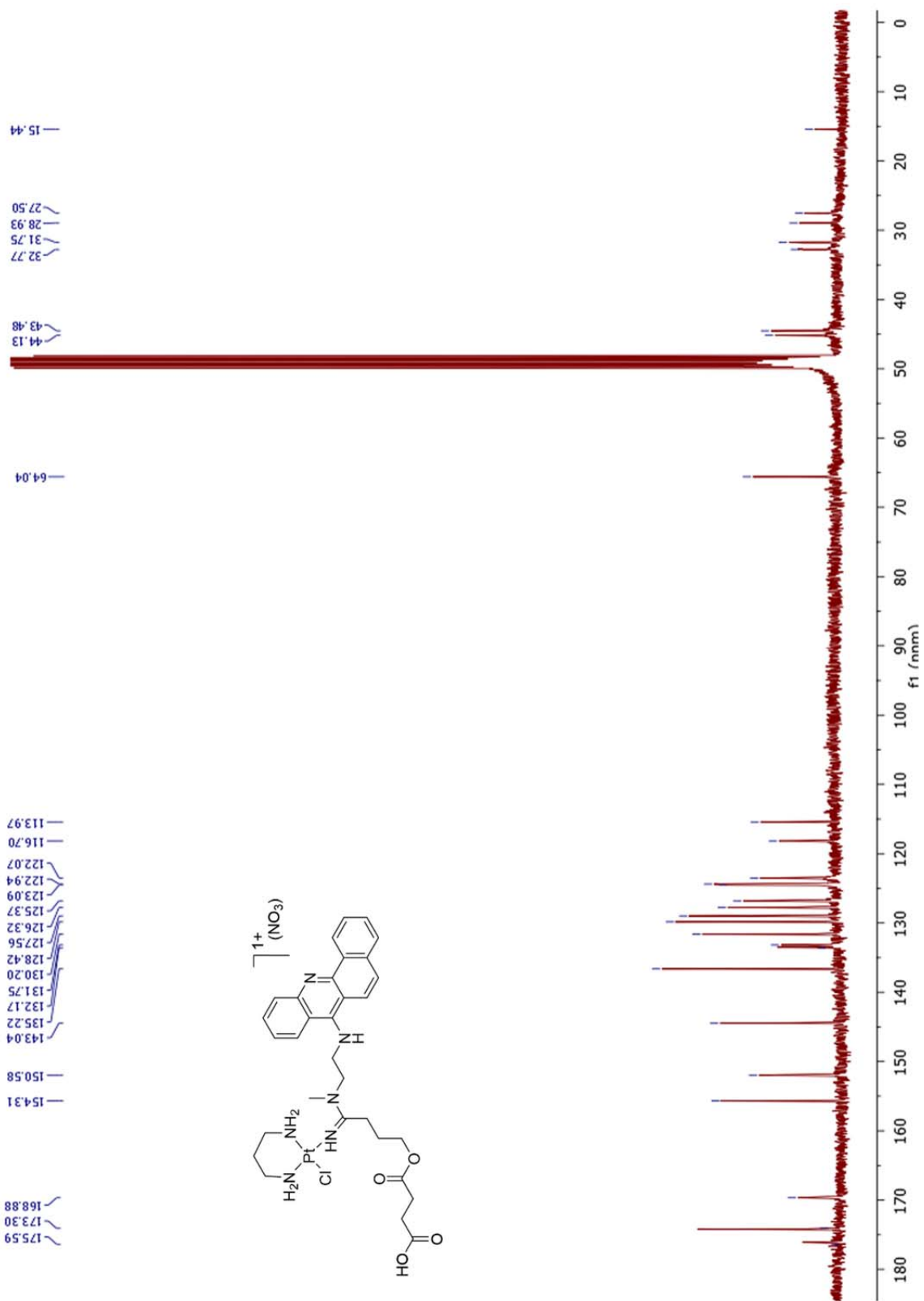
**Figure S1.**  $^1\text{H}$  NMR spectrum of **P1** in  $\text{MeOH-}d_4$ .



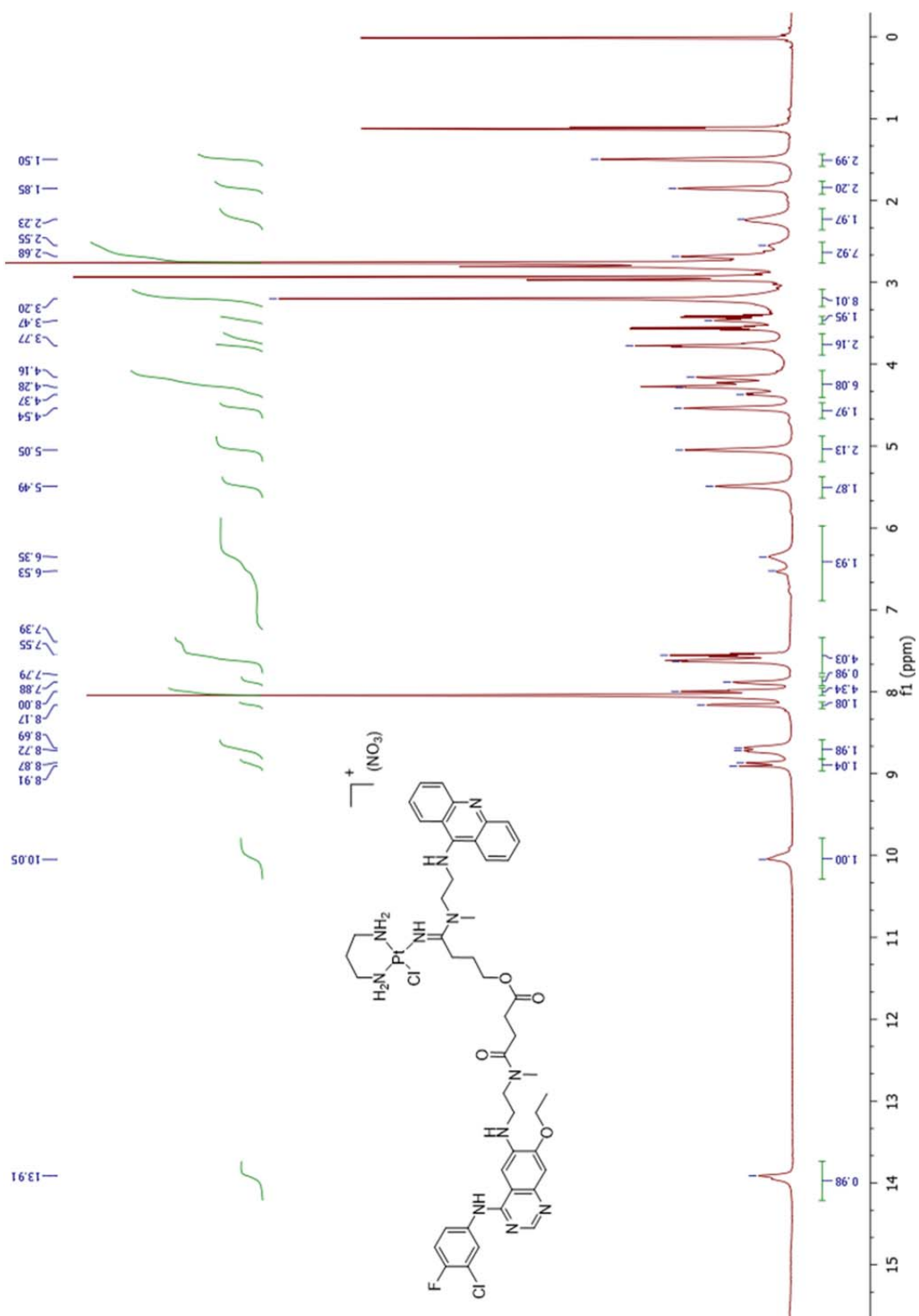
**Figure S2.**  $^{13}\text{C}$  NMR spectrum of **P1** in  $\text{MeOH-}d_4$ .



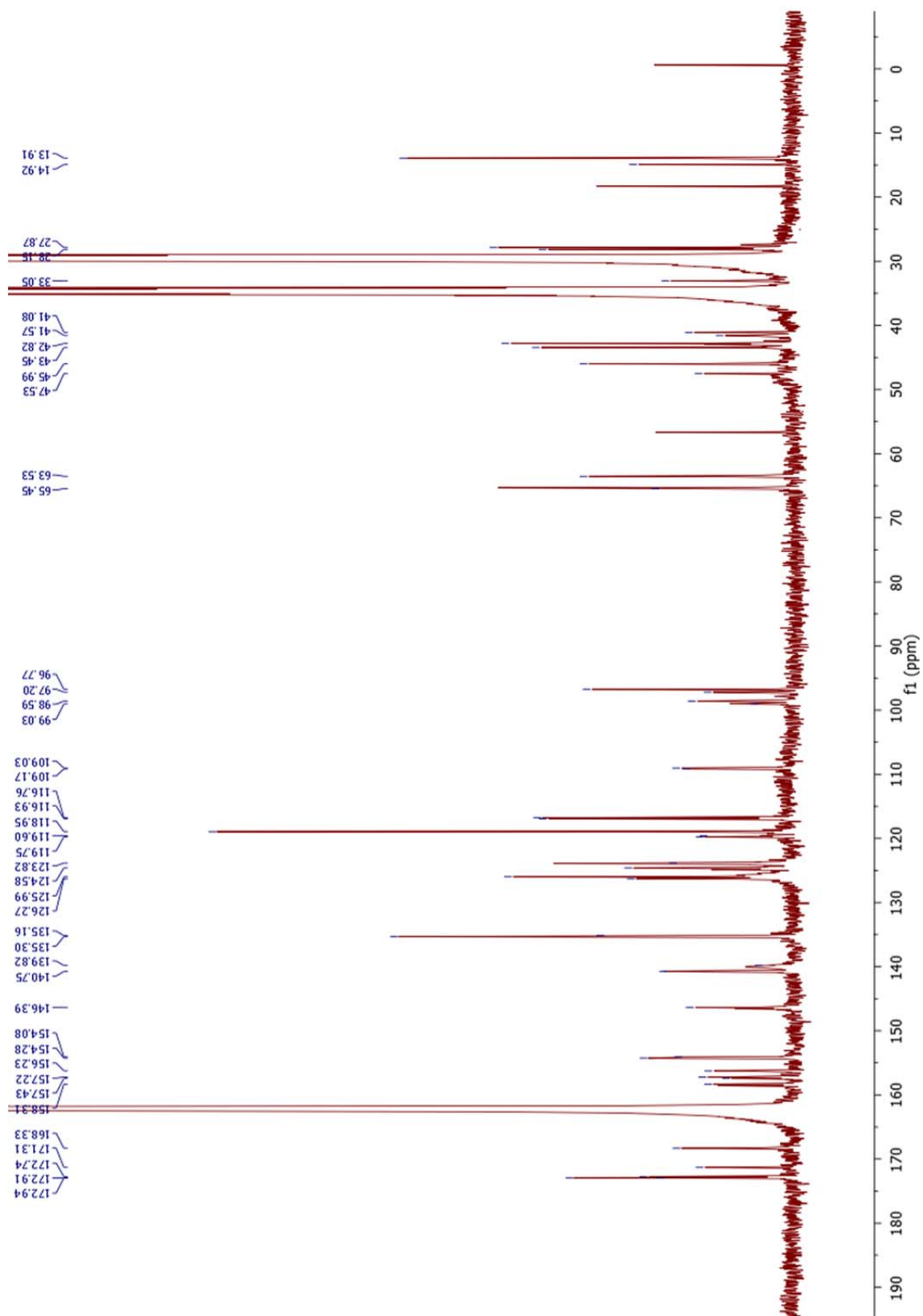
**Figure S3.**  $^1\text{H}$  NMR spectrum of **P2** in  $\text{MeOH-}d_4$ .



**Figure S4.**  $^{13}\text{C}$  NMR spectrum of P2 in  $\text{MeOH-}d_4$ .

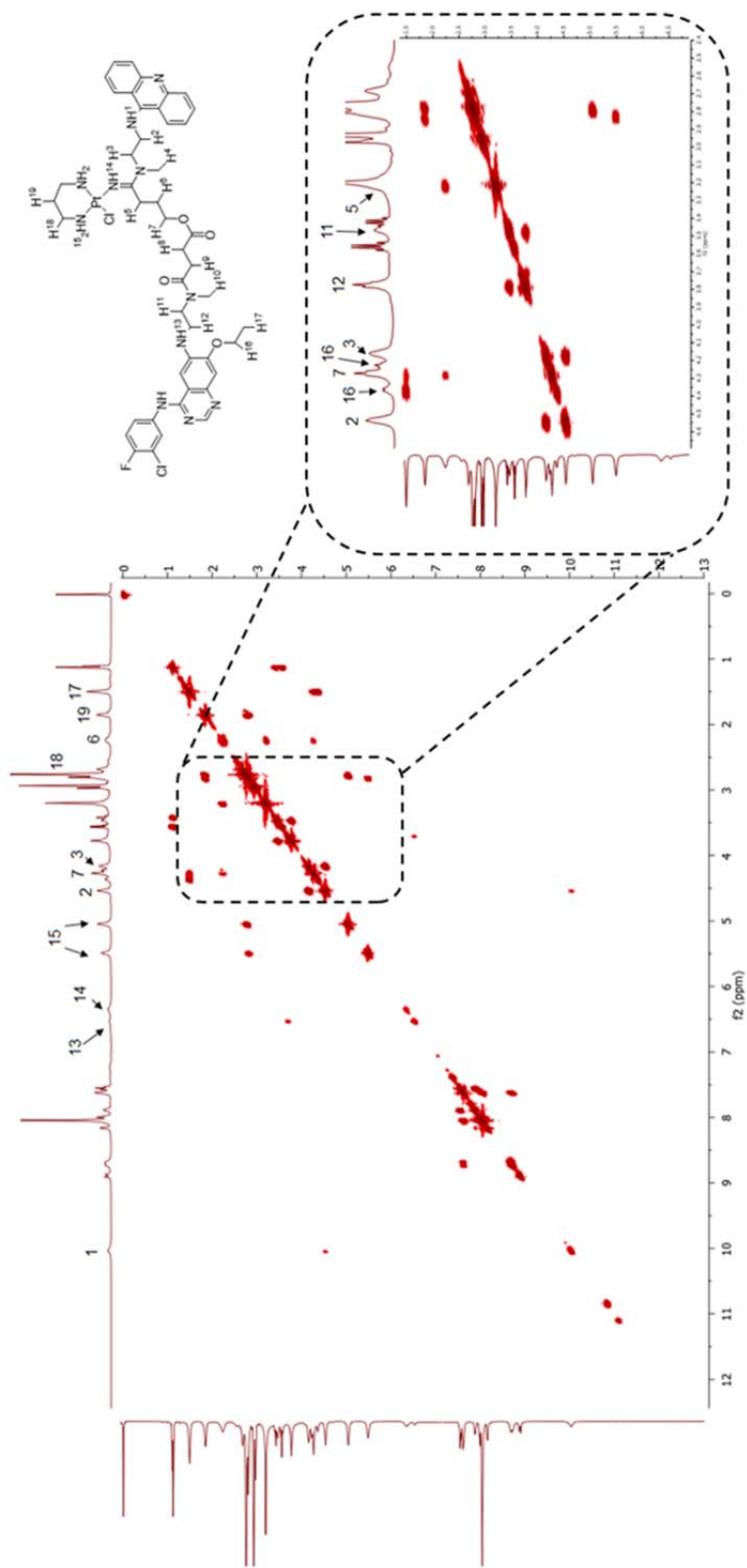


**Figure S5.**  $^1\text{H}$  NMR spectrum of **P1-N7** in  $\text{DMF-}d_7$ .

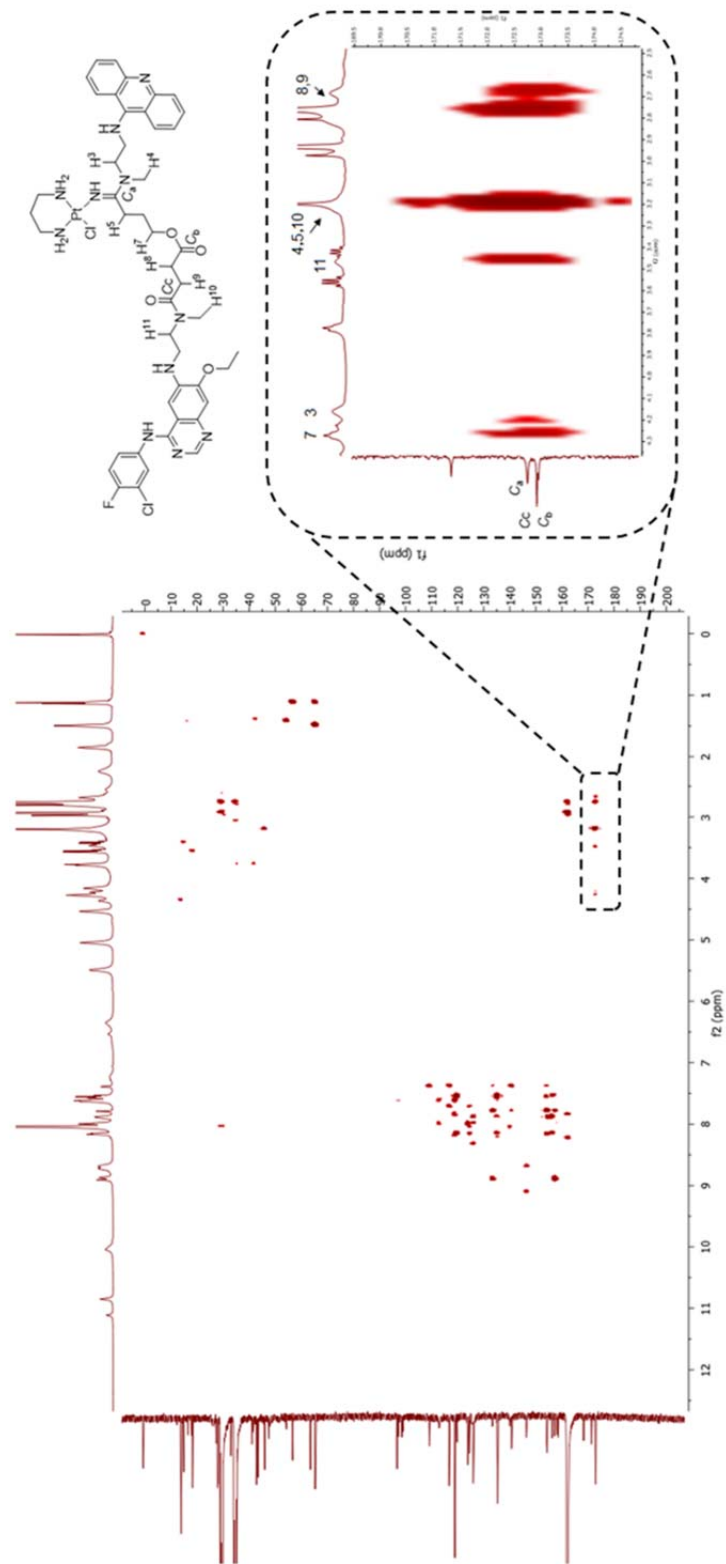


**Figure S6.**  $^{13}\text{C}$  NMR spectrum of P1-N7 in  $\text{DMF-}d_7$ .



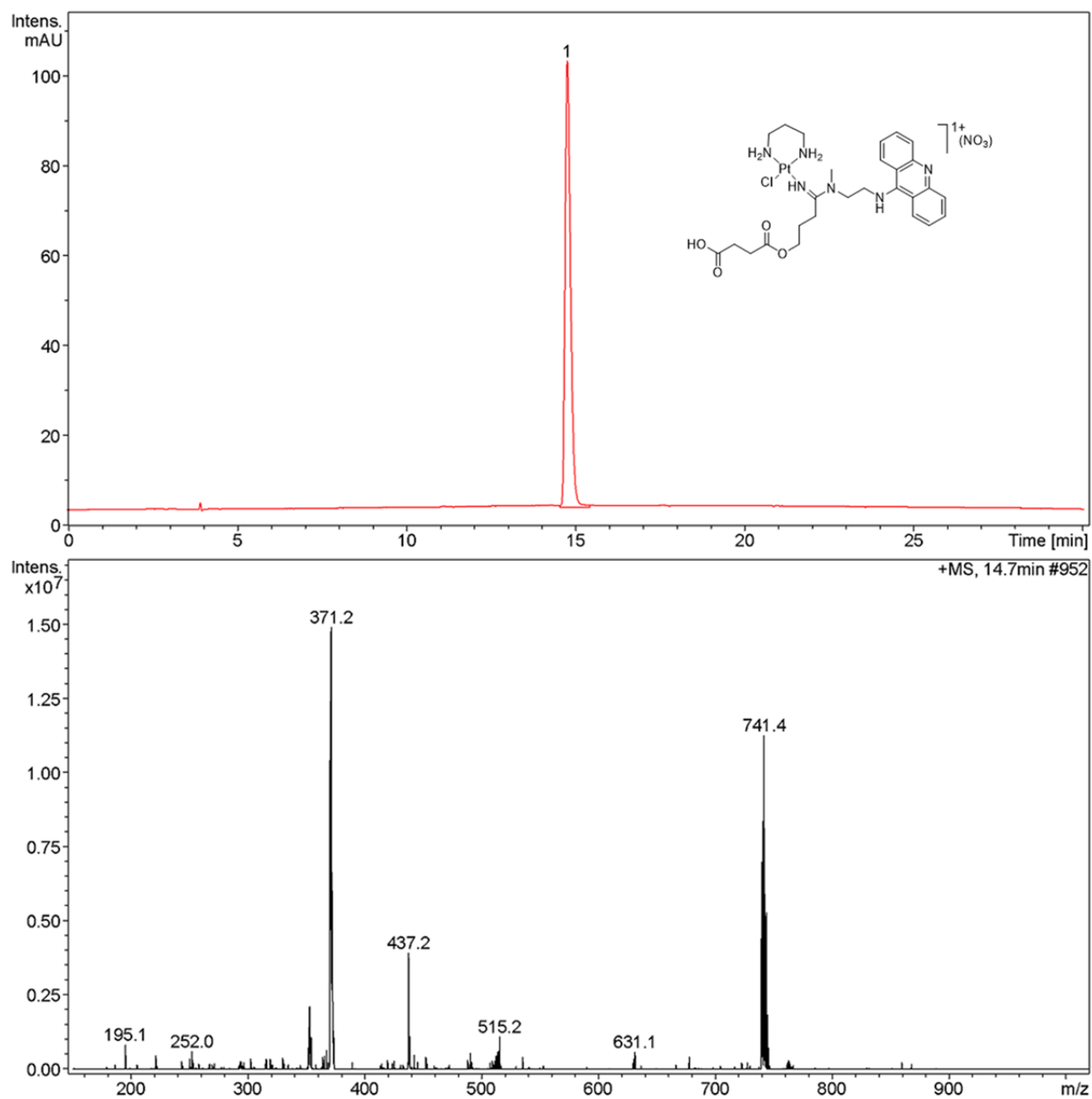


**Figure S7.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound **P1-N7** in  $\text{DMF-}d_7$ .

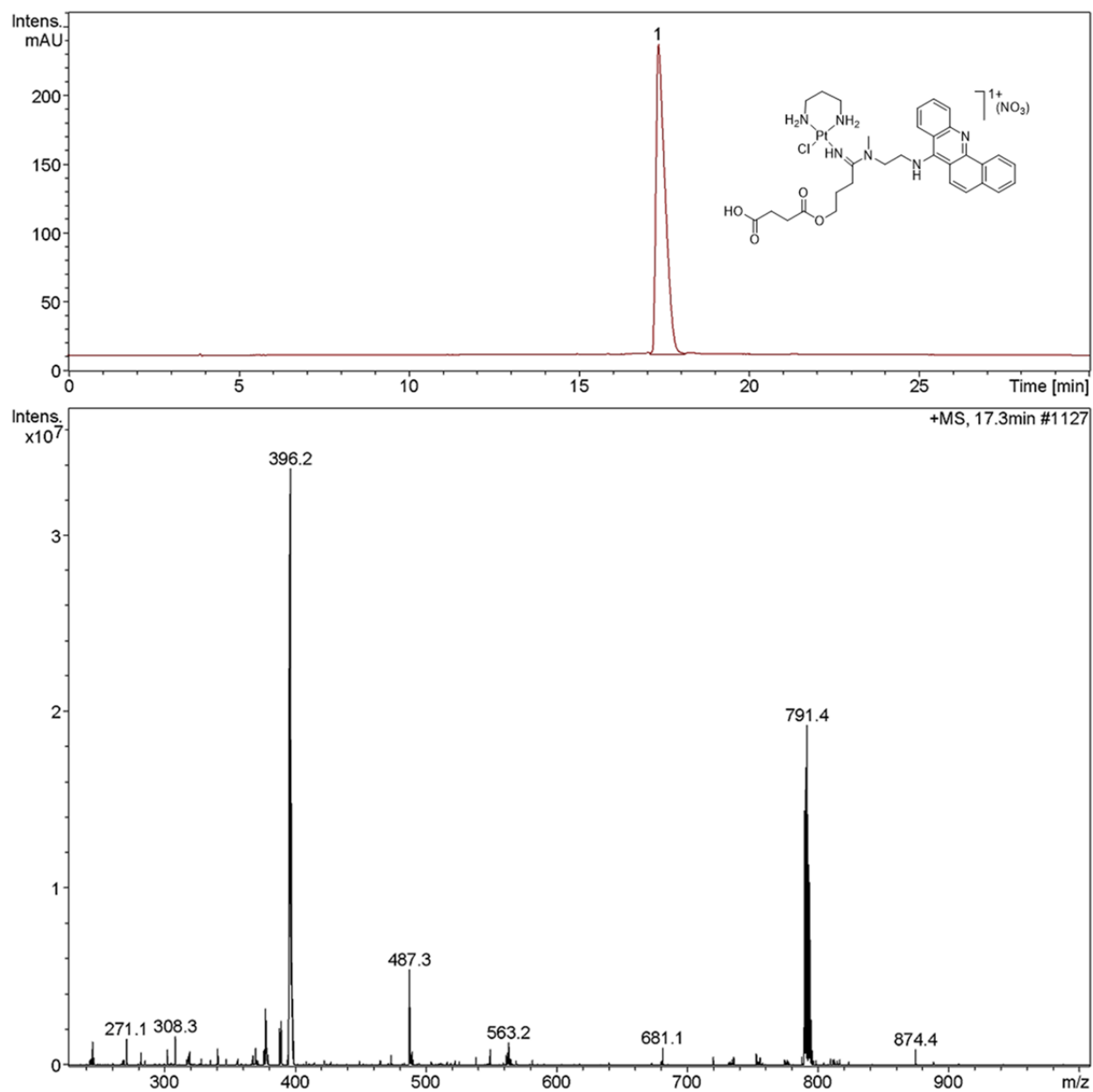


**Figure S8.**  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of compound P1-N7 in  $\text{DMF-}d_7$ .

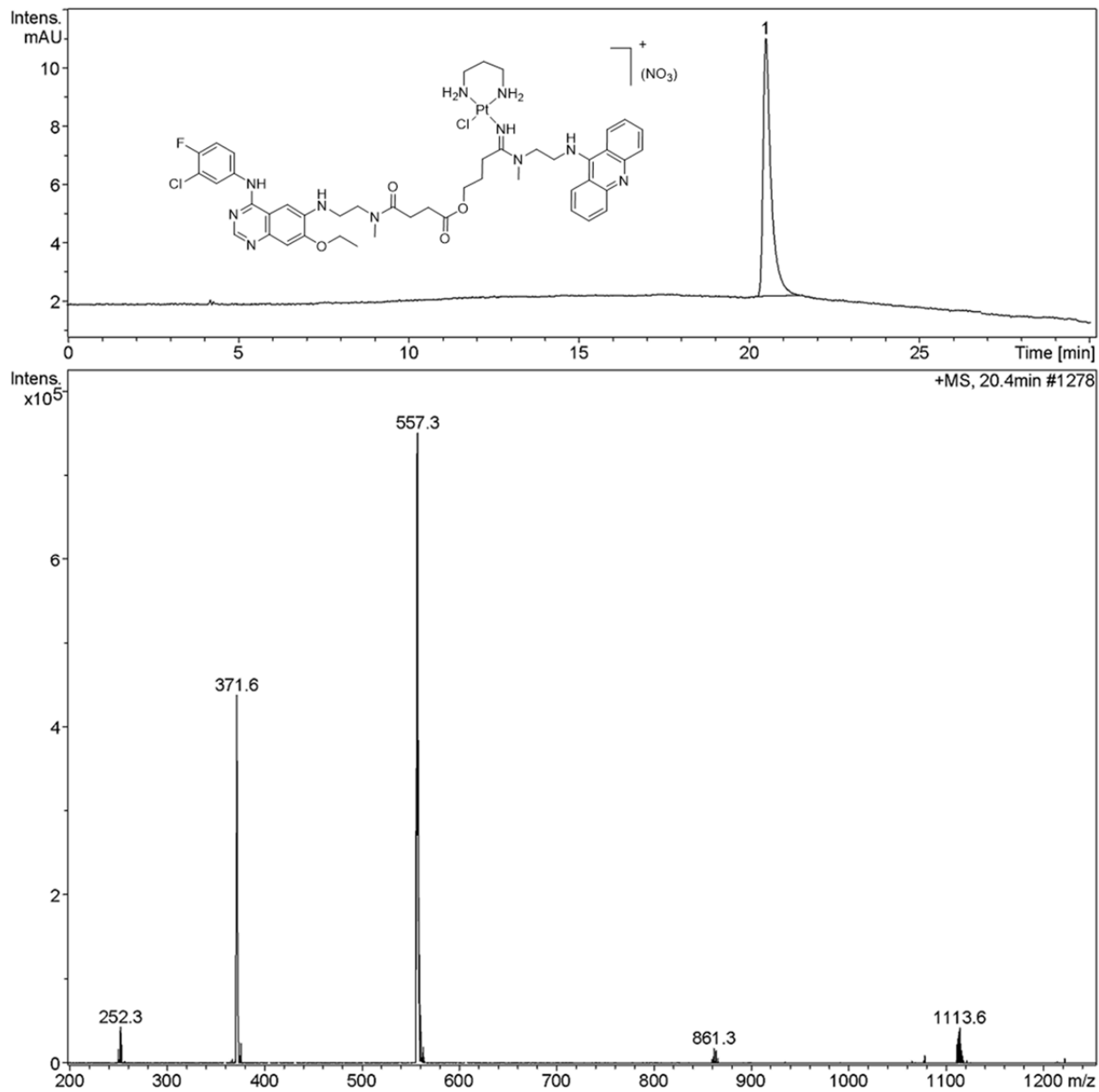
## S2. LC-MS ANALYSIS OF PURIFIED COMPOUNDS



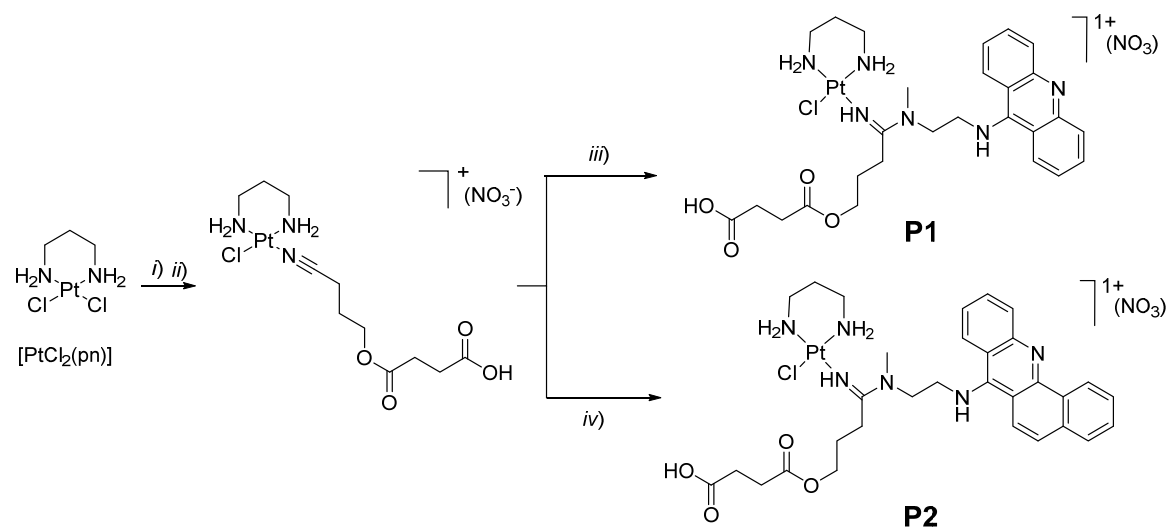
**Figure S9.** LC-MS analysis of purified **P1**.



**Figure S10.** LC-MS analysis of purified **P2**.

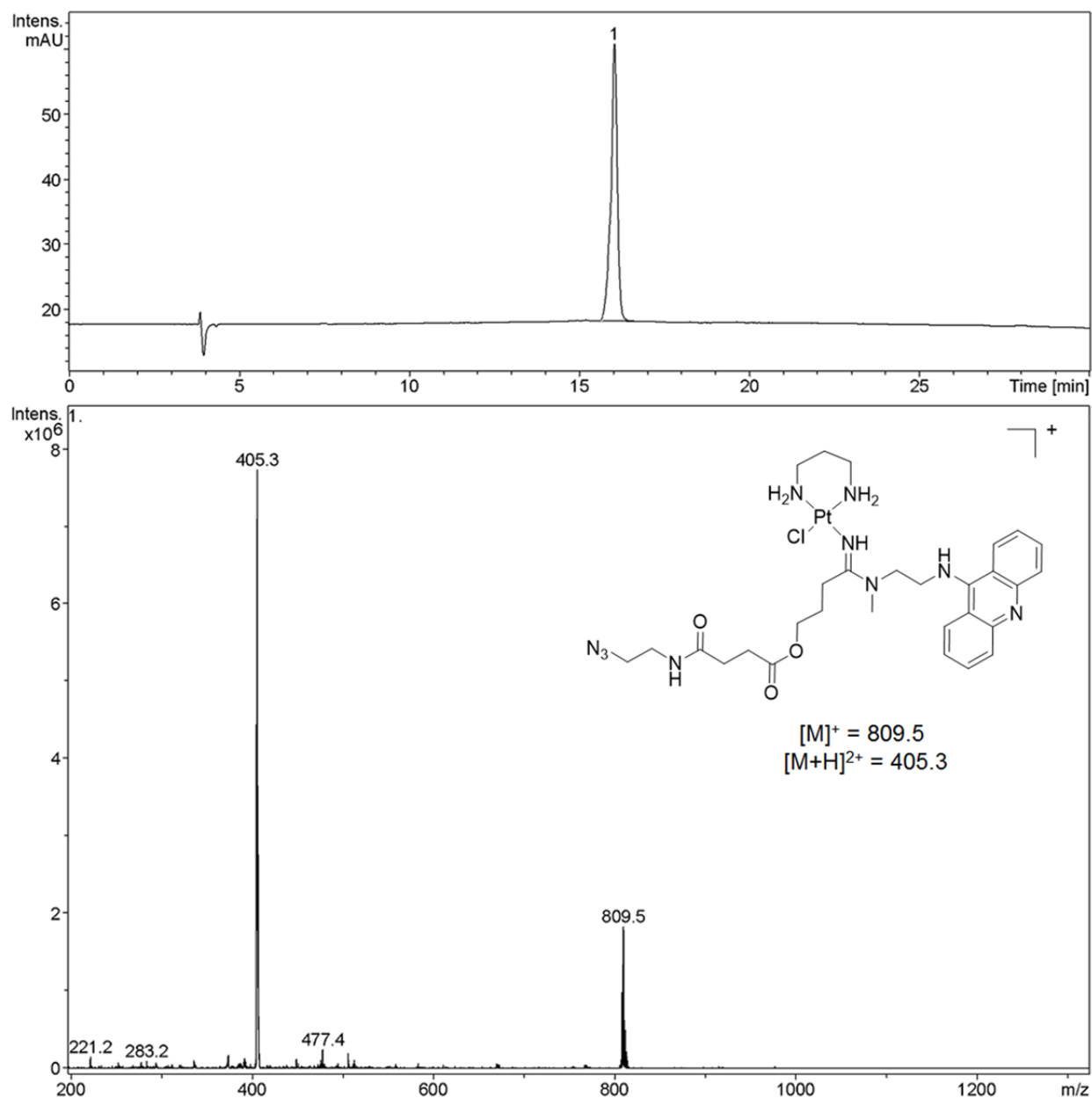


**Figure S11.** LC-MS analysis of purified P1-N7.

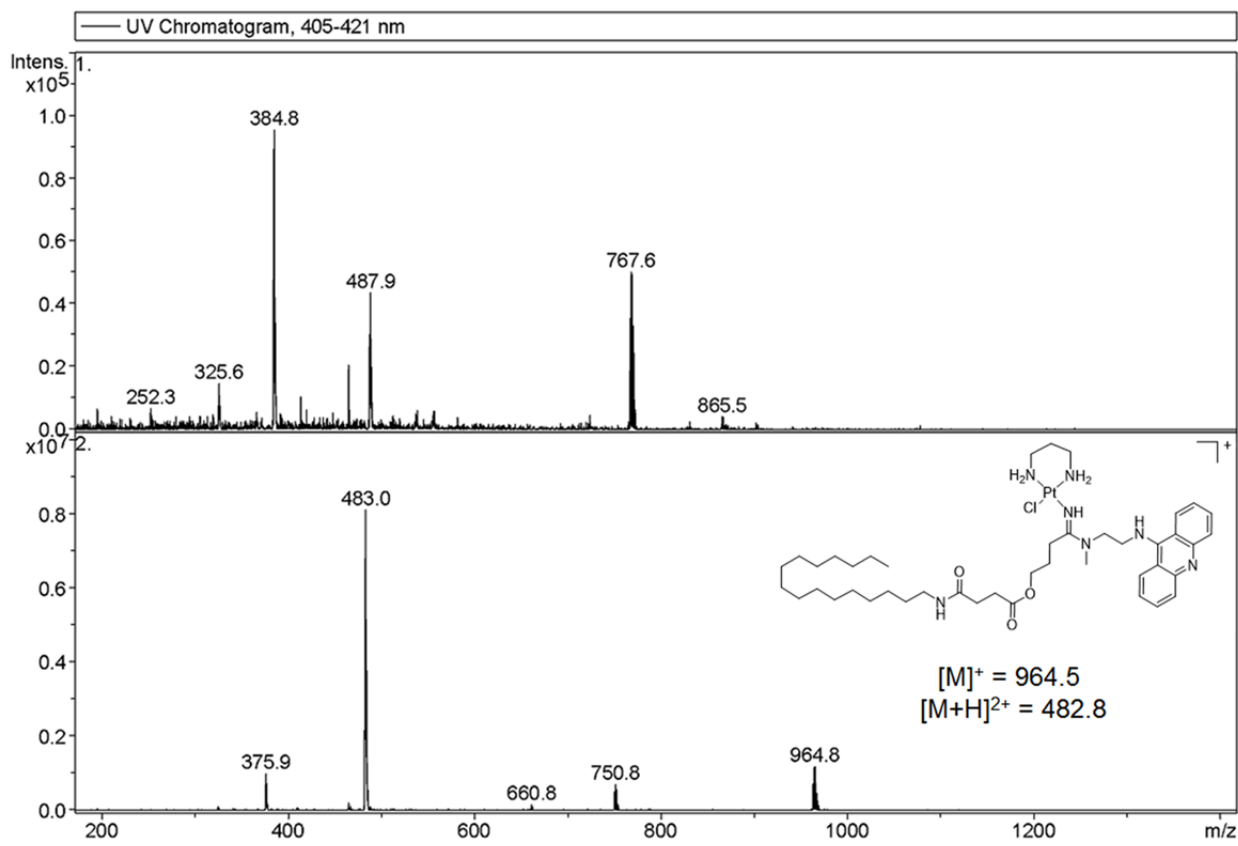
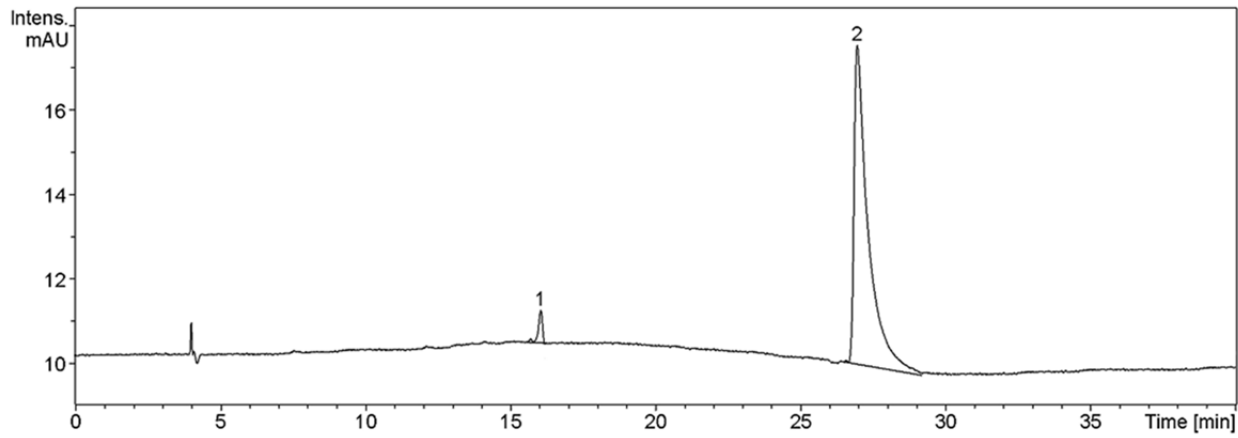


**Figure S12.** Synthesis of **P1** and **P2**. i)  $\text{AgNO}_3$ , DMF, rt, ii)  $\text{HOOC}(\text{CH}_2)_2\text{C}(\text{O})\text{O}(\text{CH}_2)_3\text{CN}$  (**3**), DMF,  $60^\circ\text{C}$ , 4 h, iii)  $\text{N}^1$ -(acridin-9-yl)- $\text{N}^2$ -methylethane-1,2-diamine (**A1**), DMF,  $4^\circ\text{C}$ , iv)  $\text{N}^1$ -(benzo[*c*]acridin-7-yl)- $\text{N}^2$ -methylethane-1,2-diamine (**B1**), DMF,  $4^\circ\text{C}$ .

### S3. LC-MS ANALYSIS OF COUPLING REACTIONS

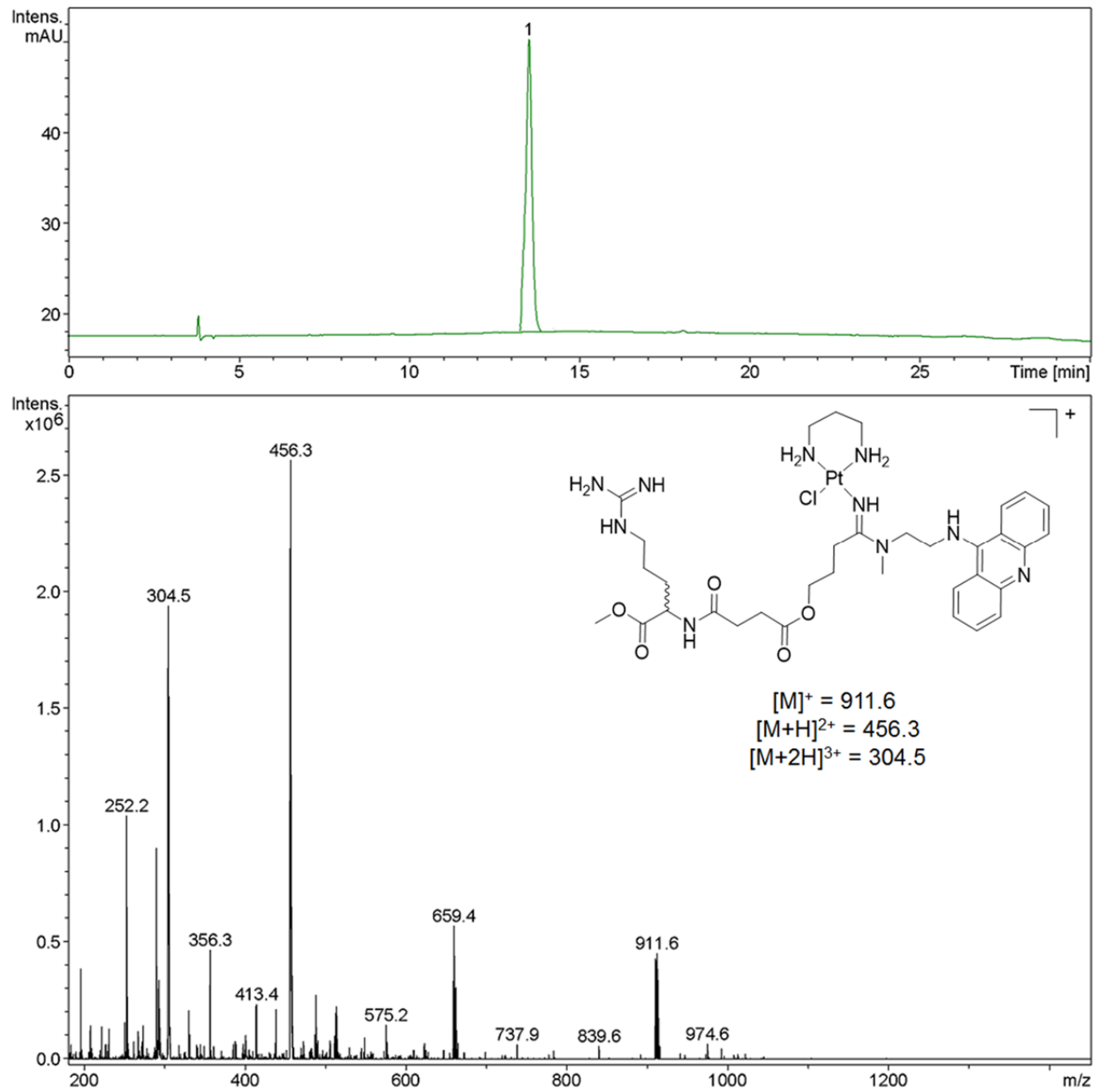


**Figure S13.** LC-MS analysis of the reaction mixture for the preparation of **P1-N1**.

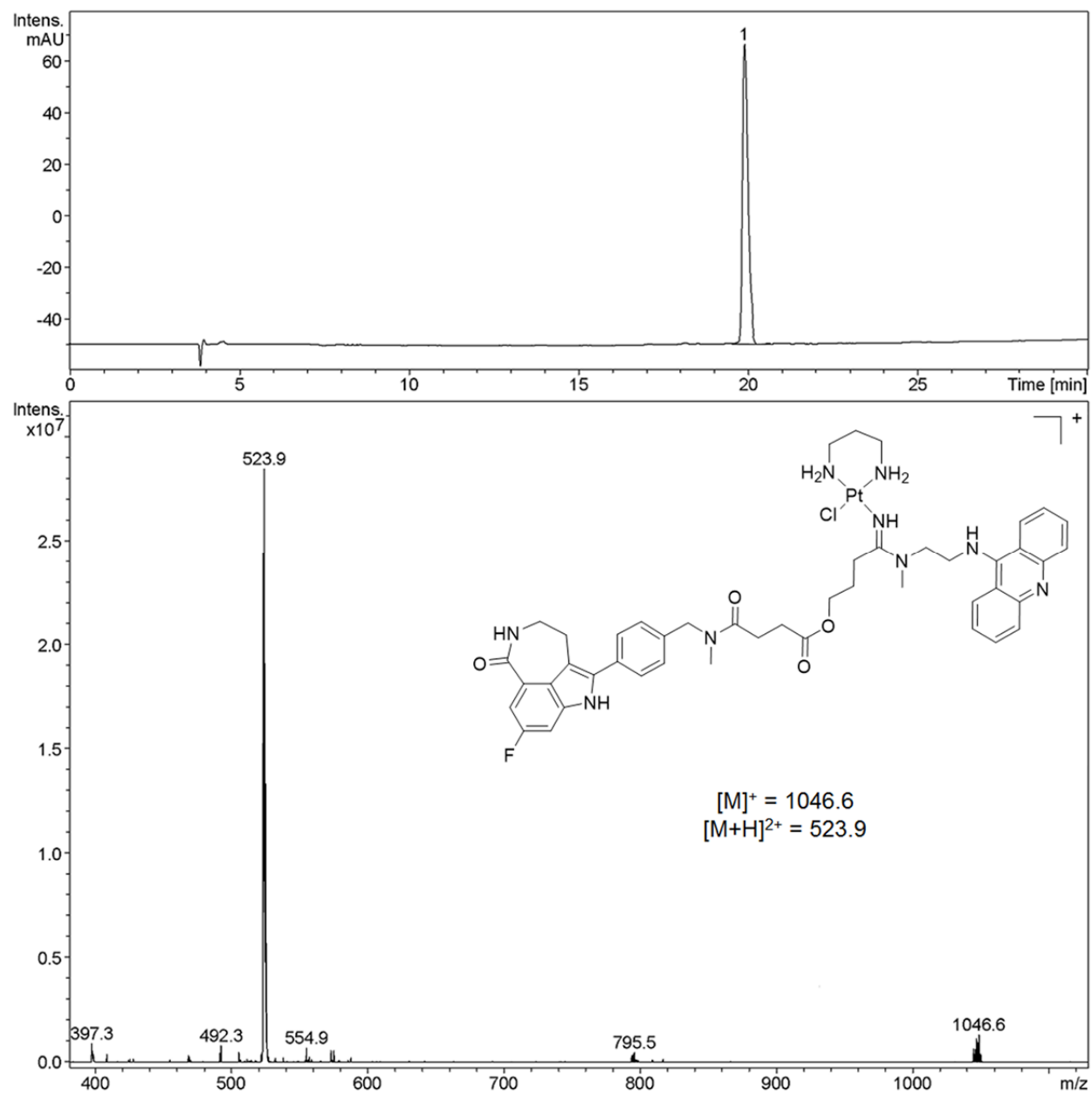


**Figure S14.** LC-MS analysis of the reaction mixture for the preparation of **P1-N2**.

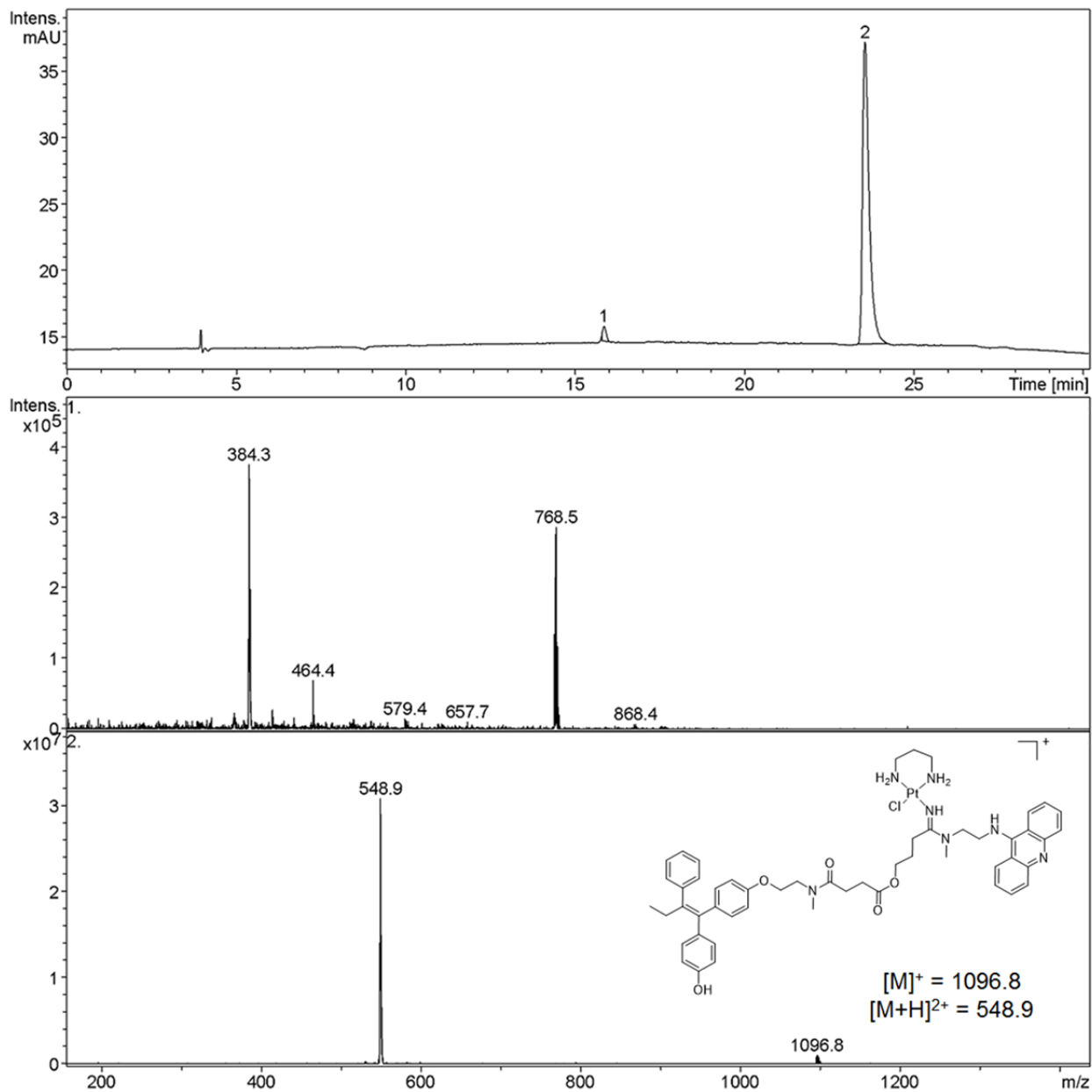




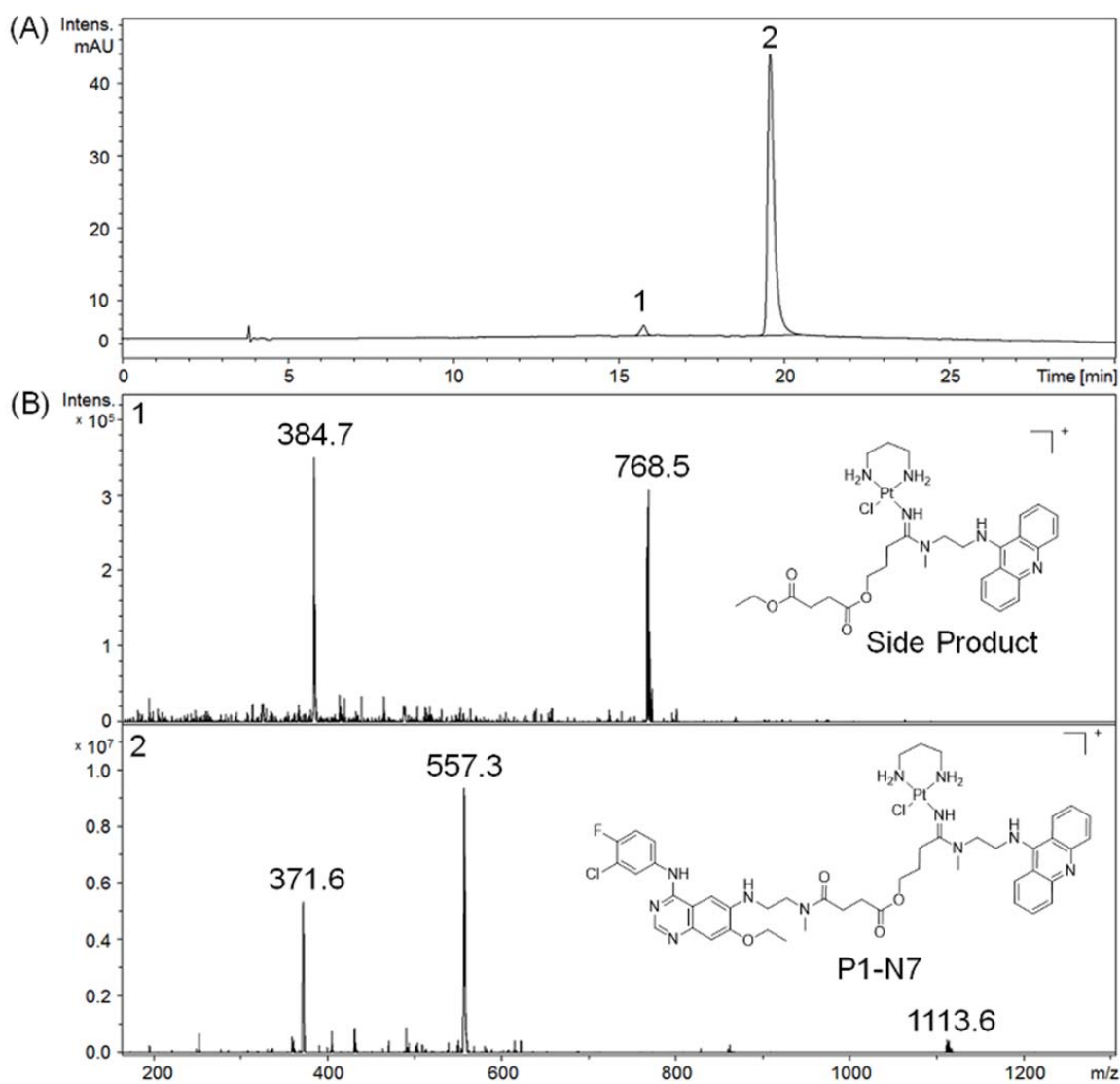
**Figure S15.** LC-MS analysis of the reaction mixture for the preparation of P1-N4.



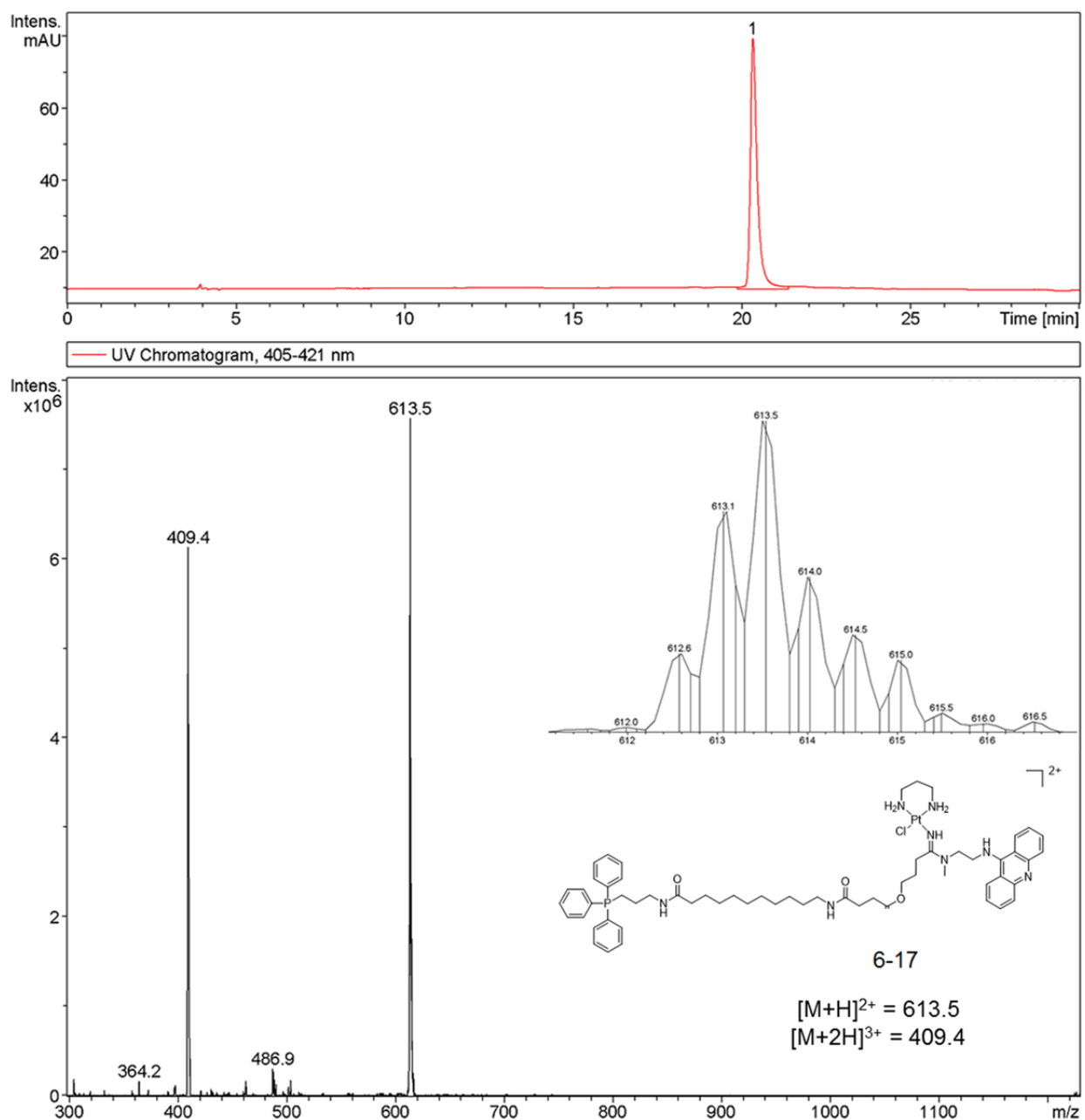
**Figure S16.** LC-MS analysis of the reaction mixture for the preparation of **P1-N5**.



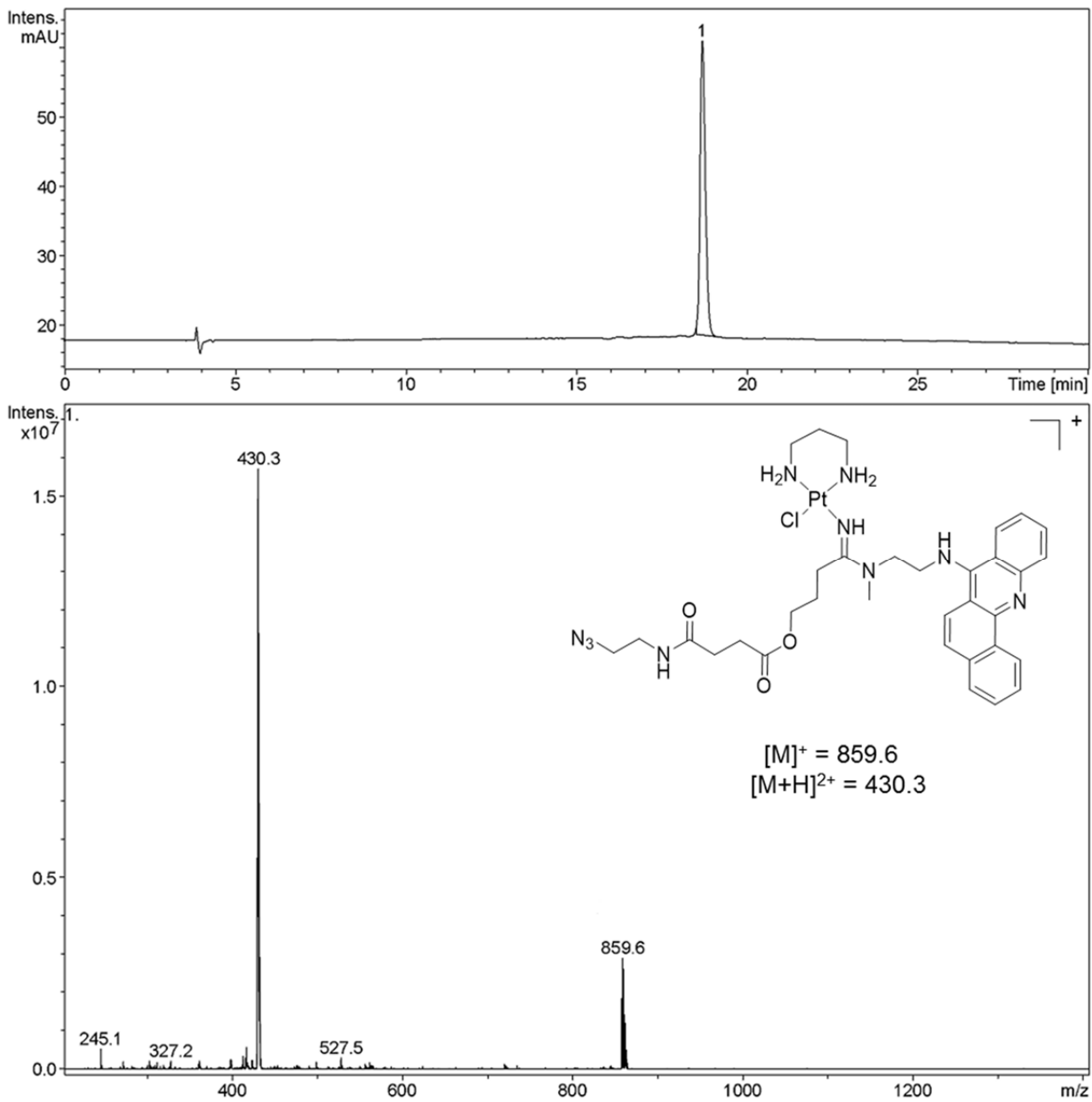
**Figure S17.** LC-MS analysis of the reaction mixture for the preparation of P1-N6.



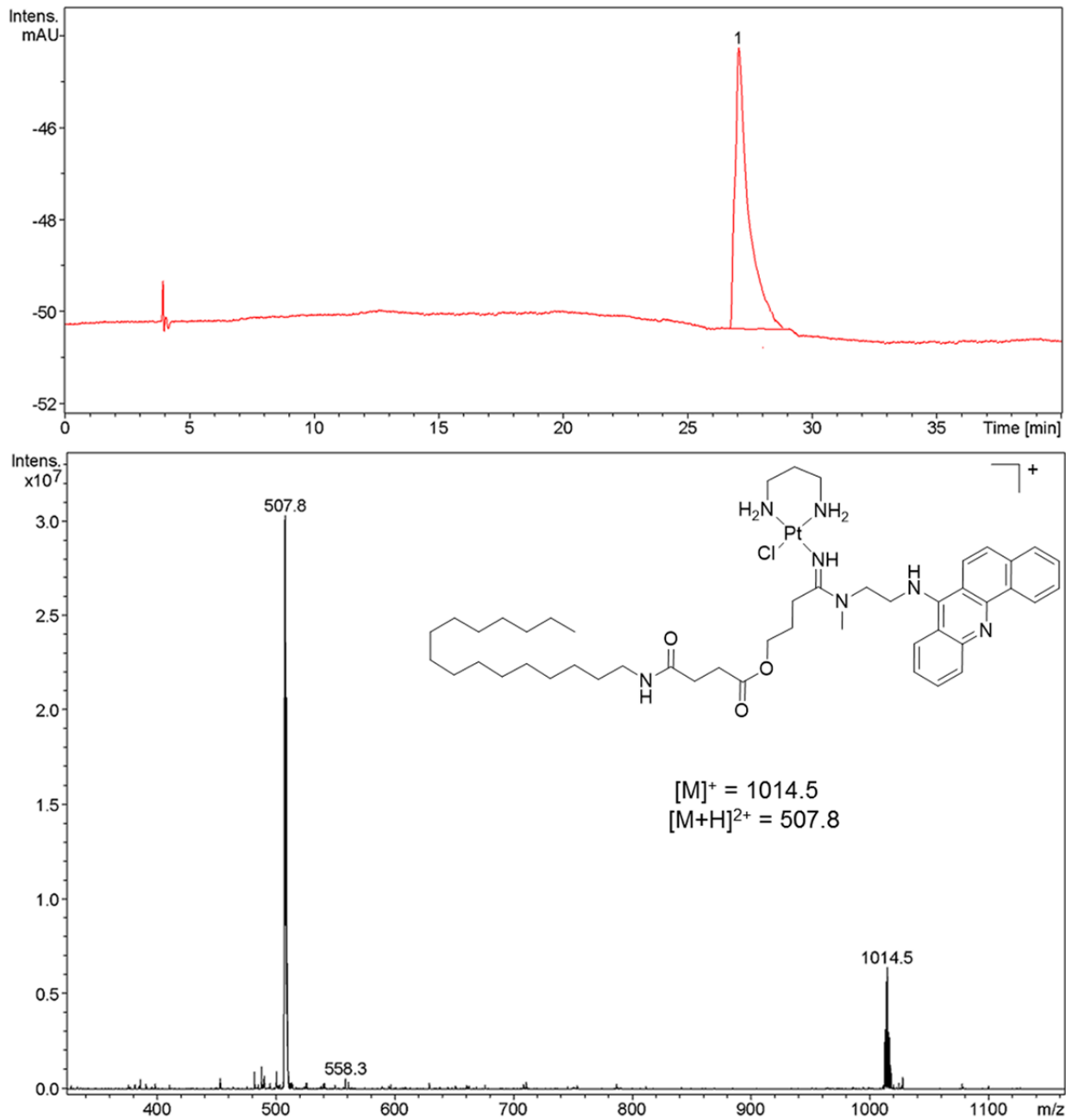
**Figure S18.** (A) Reverse-phase HPLC trace for the reaction of **P1** and **N7**. (B) ESMS spectrum recorded in positive-ion mode. Characteristic molecular and fragment ions for **P1-N7** are  $m/z$   $[M]^+$  1113.6,  $[M+H]^{2+}$  557.3,  $[M+2H]^{3+}$  371.6.



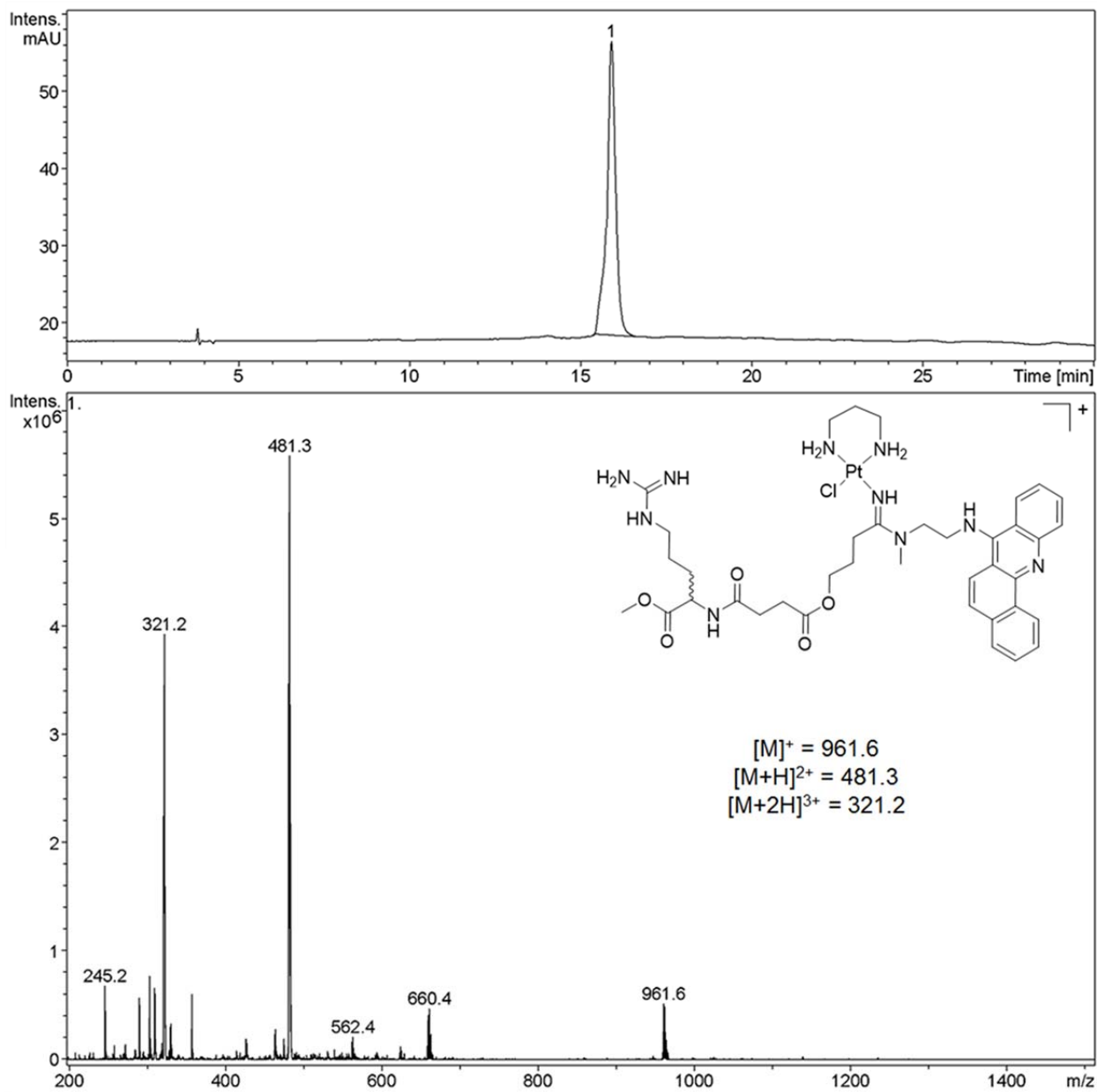
**Figure S19.** LC-MS analysis of the reaction mixture for the preparation of **P1-N9**.



**Figure S20.** LC-MS analysis of the reaction mixture for the preparation of **P2-N1**.

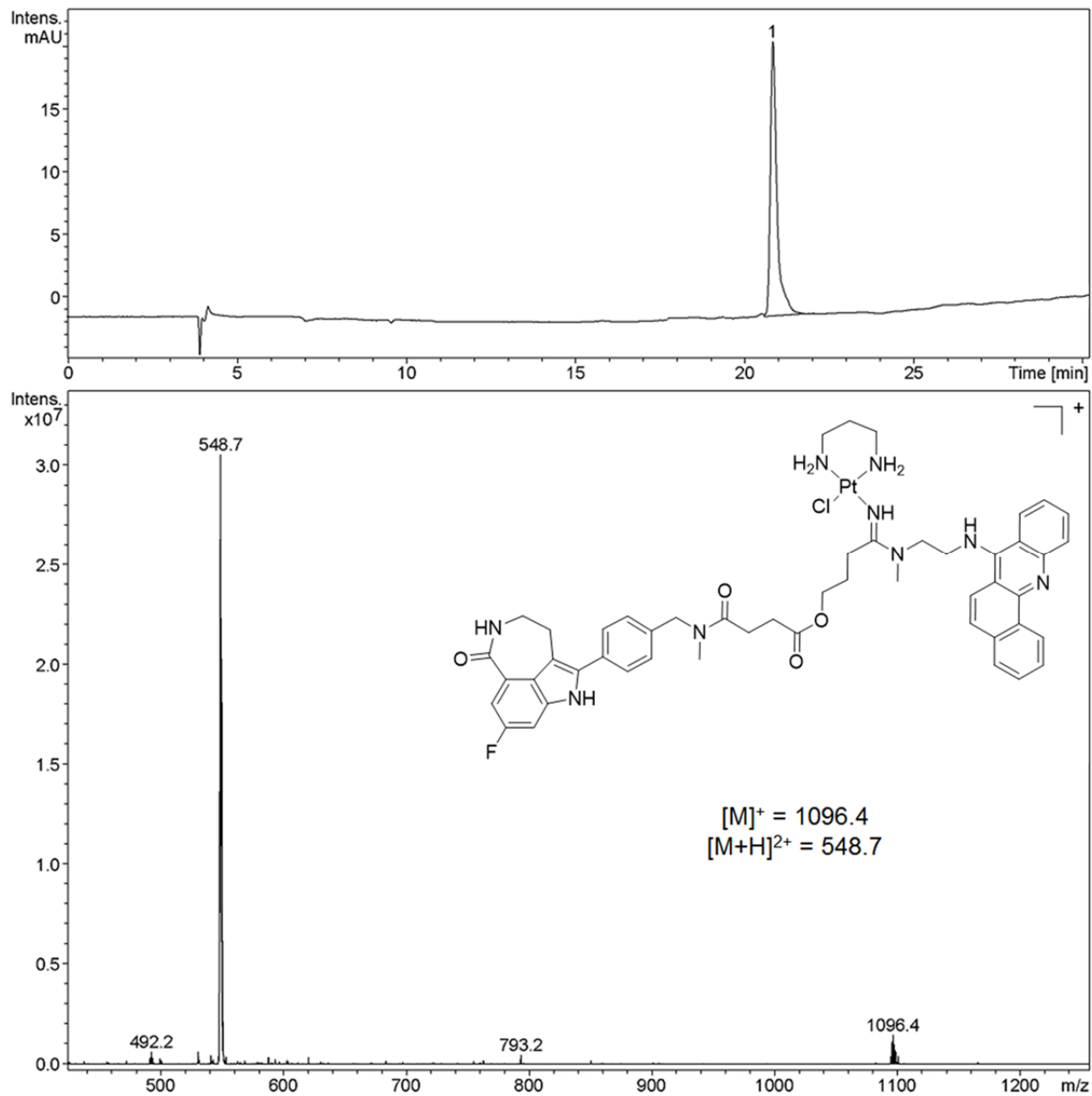


**Figure S21.** LC-MS analysis of the reaction mixture for the preparation of **P2-N2**.

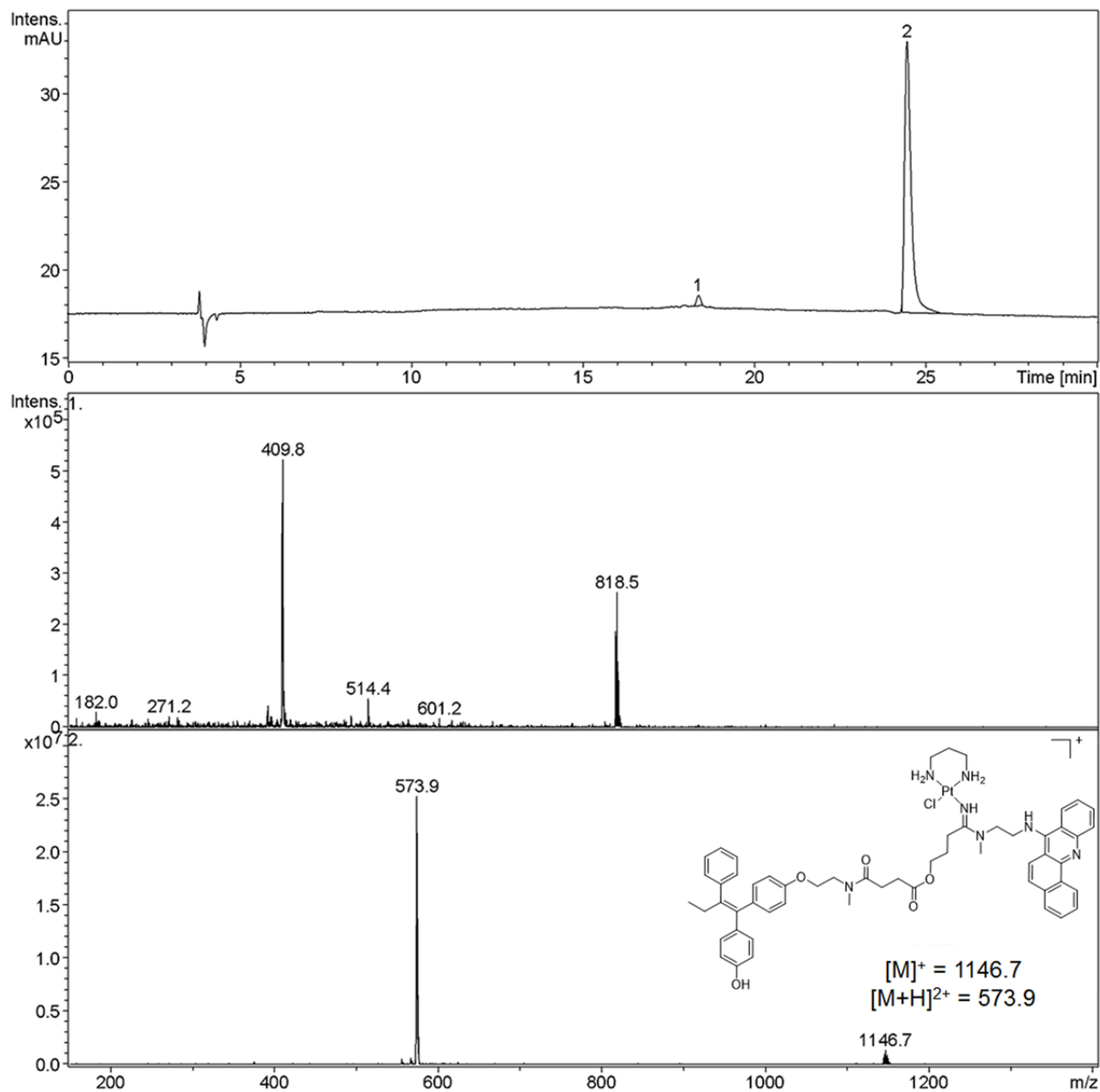


**Figure S22.** LC-MS analysis of the reaction mixture for the preparation of P2-N4.

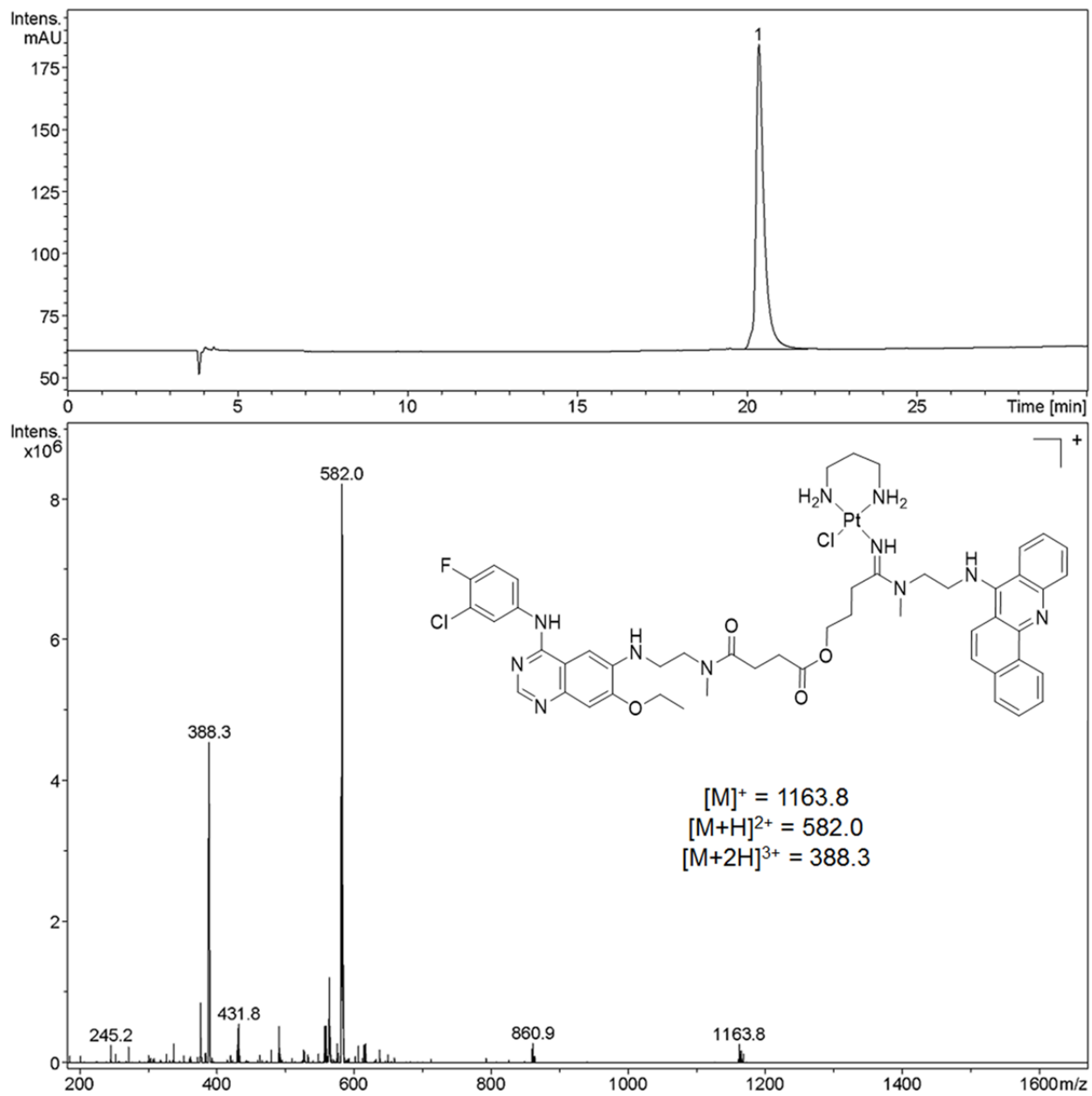




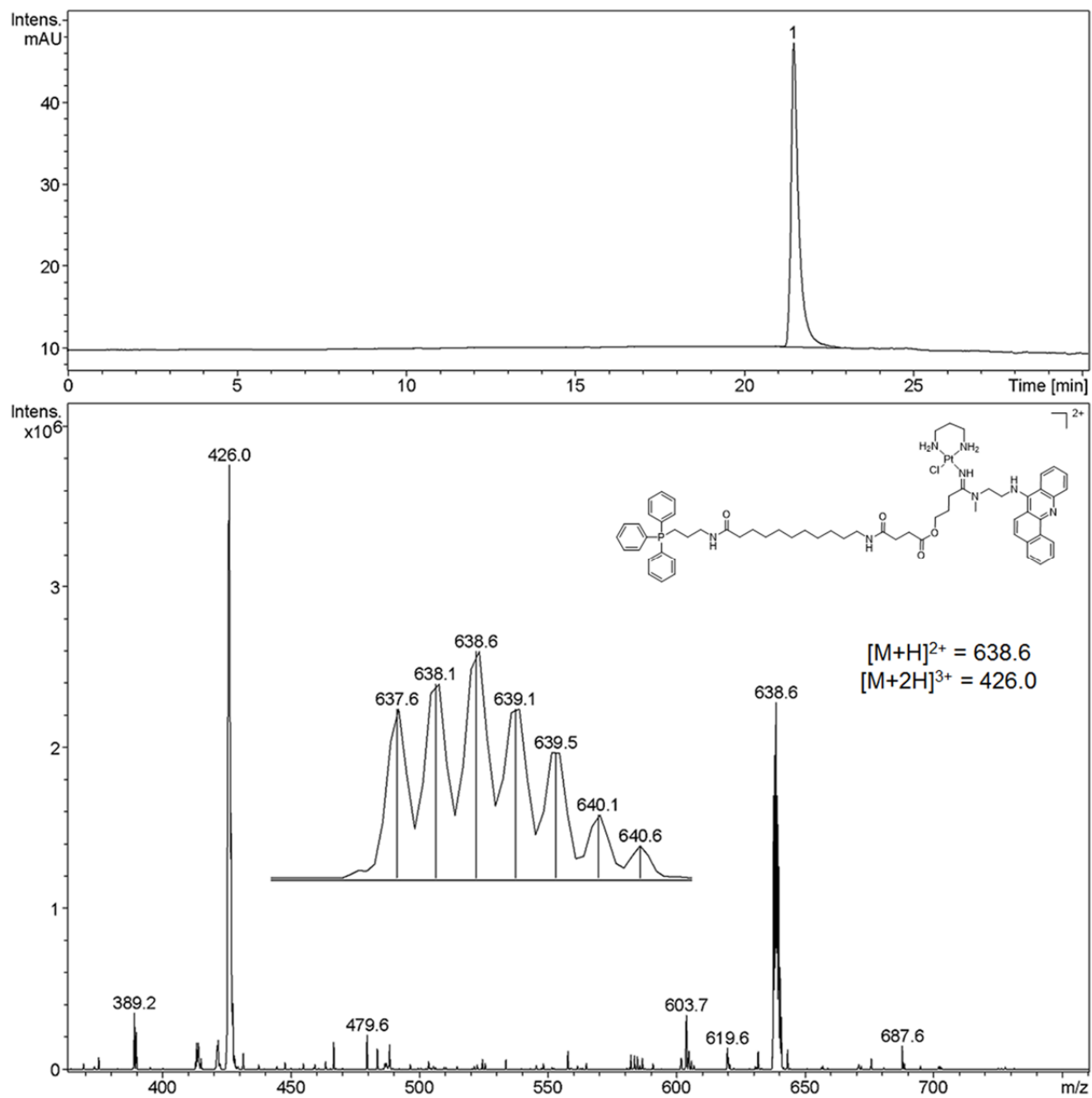
**Figure S23.** LC-MS analysis of the reaction mixture for the preparation of **P2-N5**.



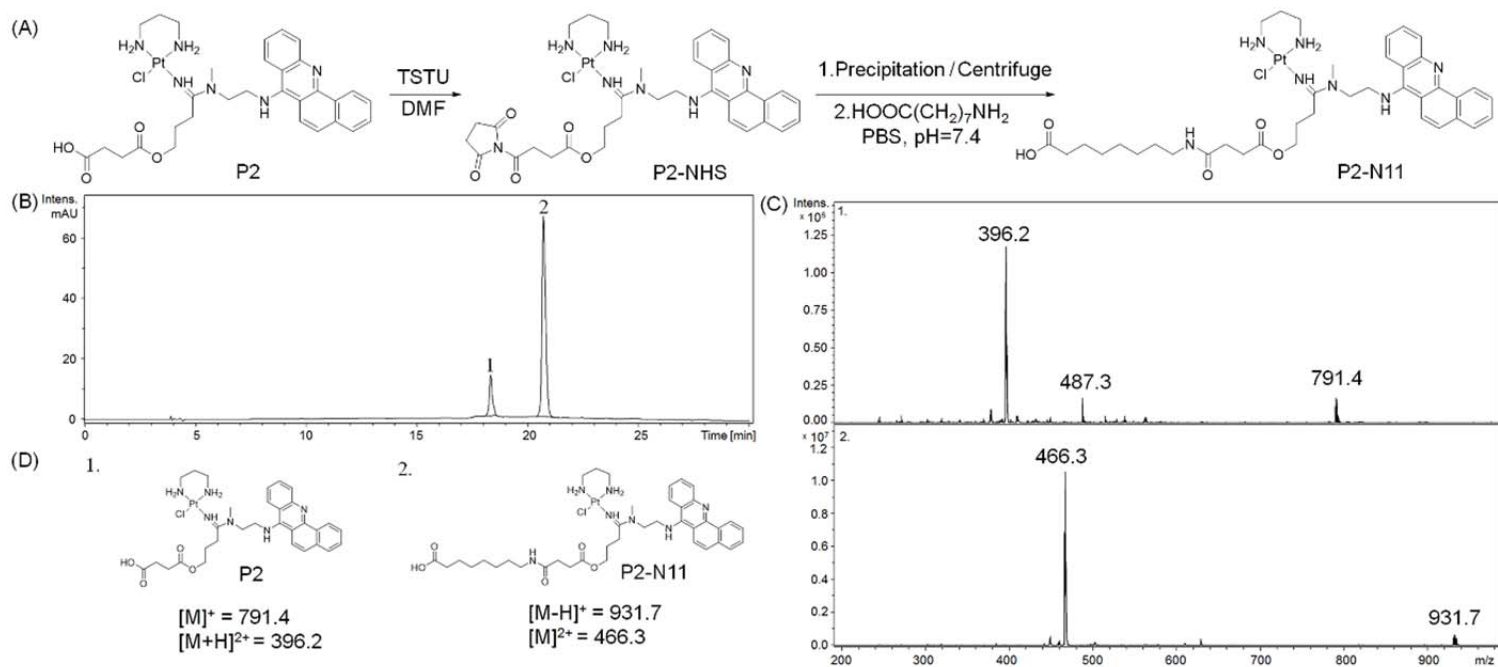
**Figure S24.** LC-MS analysis of the reaction mixture for the preparation of **P2-N6**.



**Figure S25.** LC-MS analysis of the reaction mixture for the preparation of **P2-N7**.

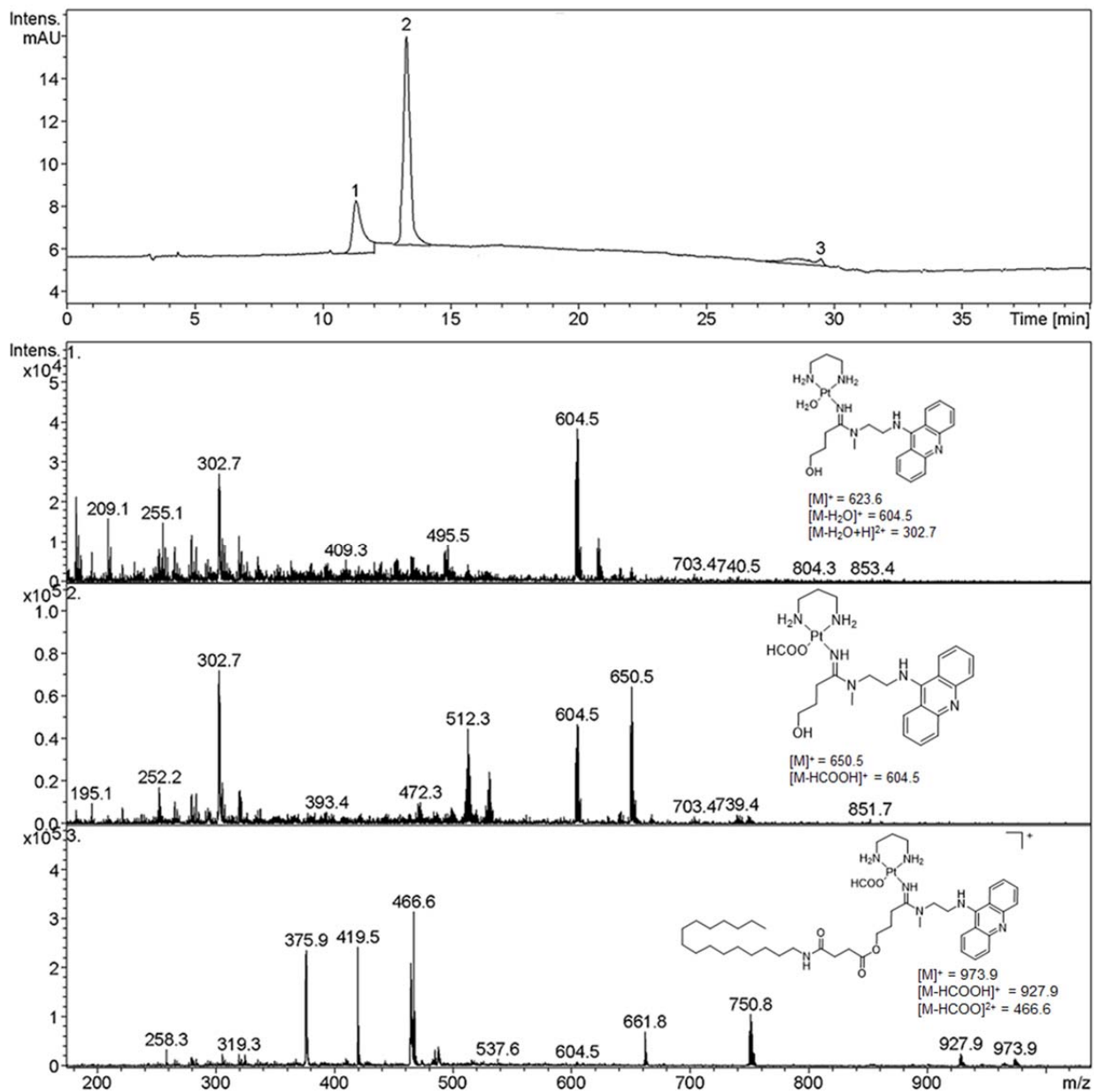


**Figure S26.** LC-MS analysis of the reaction mixture for the preparation of **P2-N9**.

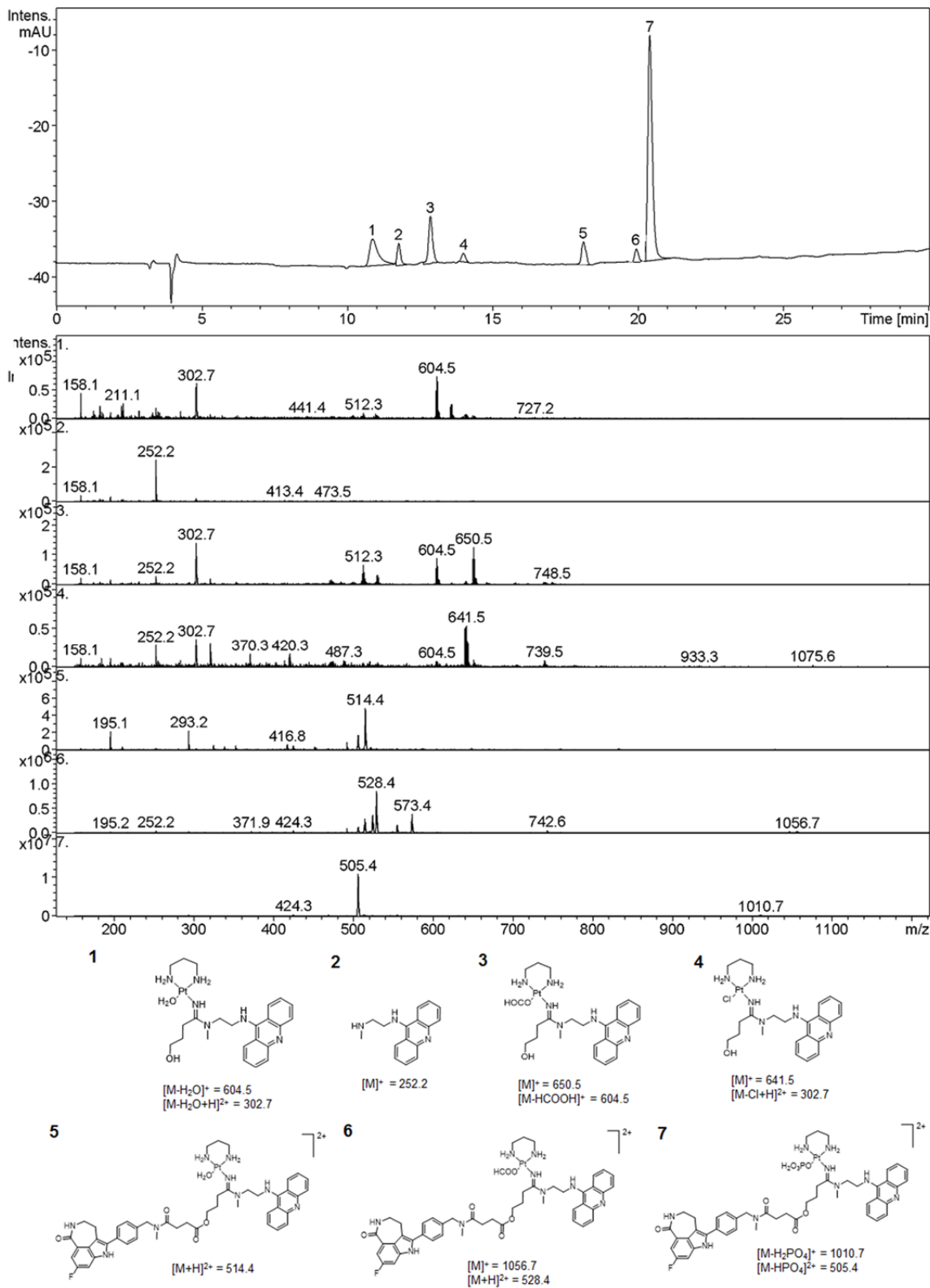


**Figure S27.** (A) “Two-step” synthesis of platinum-acridine conjugates under aqueous conditions. (B) Reverse-phase HPLC trace of the reaction mixture for the preparation of **P2-N11**. (C) ESMS spectrum of **P2** and target conjugate **P2-N11** recorded in positive-ion mode. (D) Structures and characteristic molecular ions and fragment ions ( $m/z$ ) for **P2** and **P2-N11**.

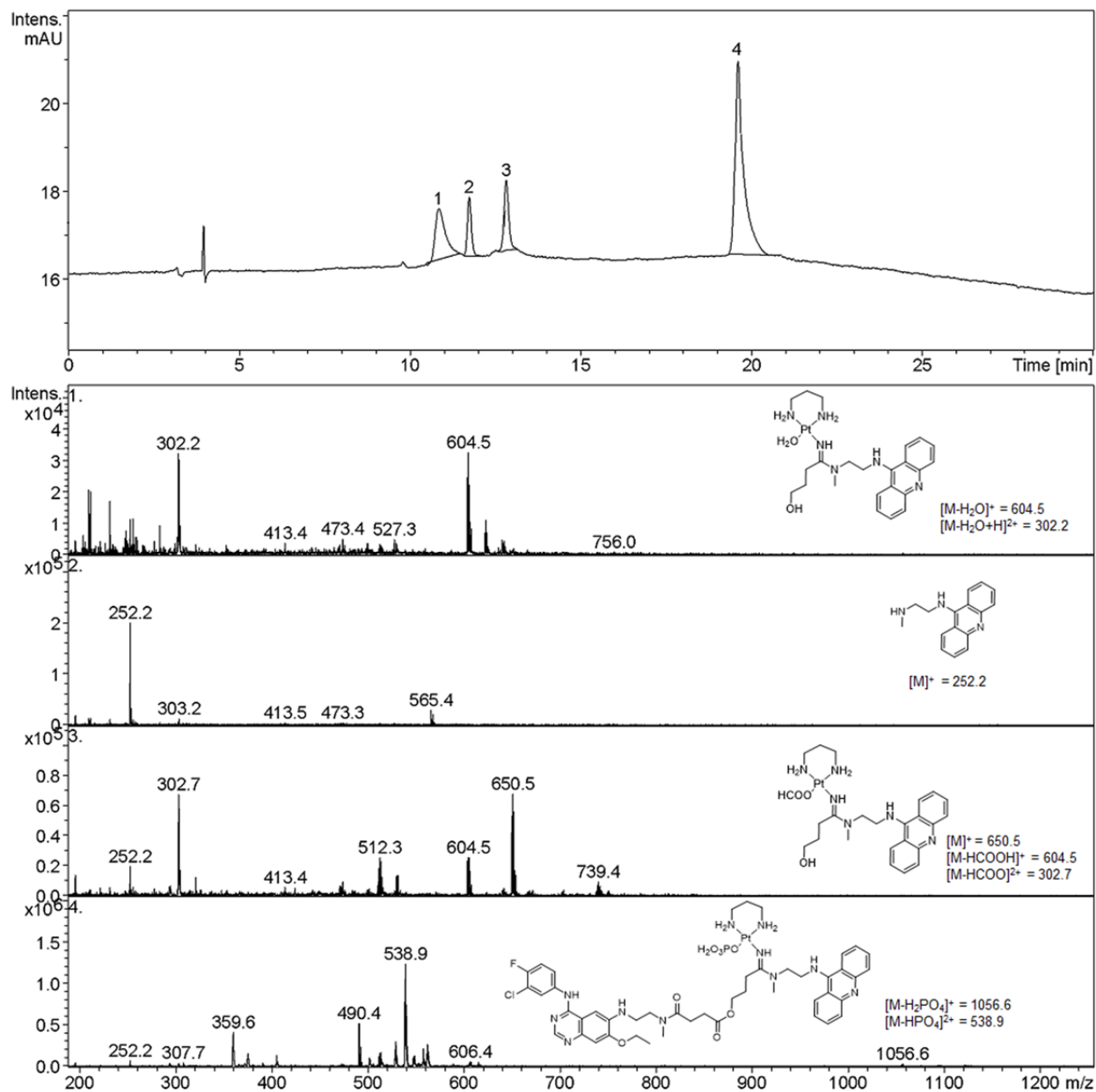
## S.4. LC-MS ANALYSIS OF ESTER REACTIVITY



**Figure S28.** LC-ESMS analysis of the mixture of compound P1-N2 in phosphate buffer (PB, pH 7.4) incubated at 37 °C.

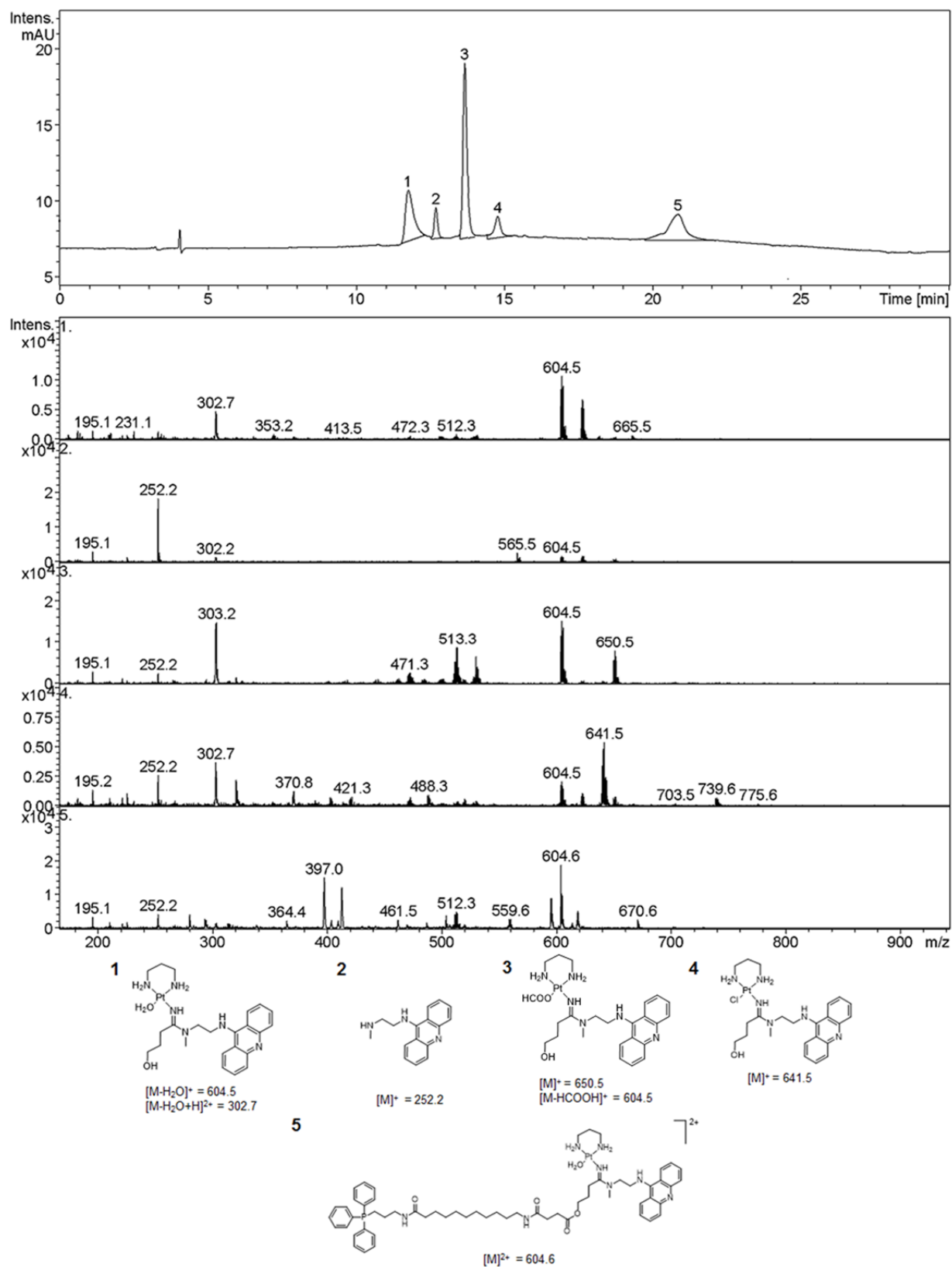


**Figure S29.** LC-ESMS analysis of the mixture of compound **P1-N5** in phosphate buffer (PB, pH 7.4) incubated at 37 °C.

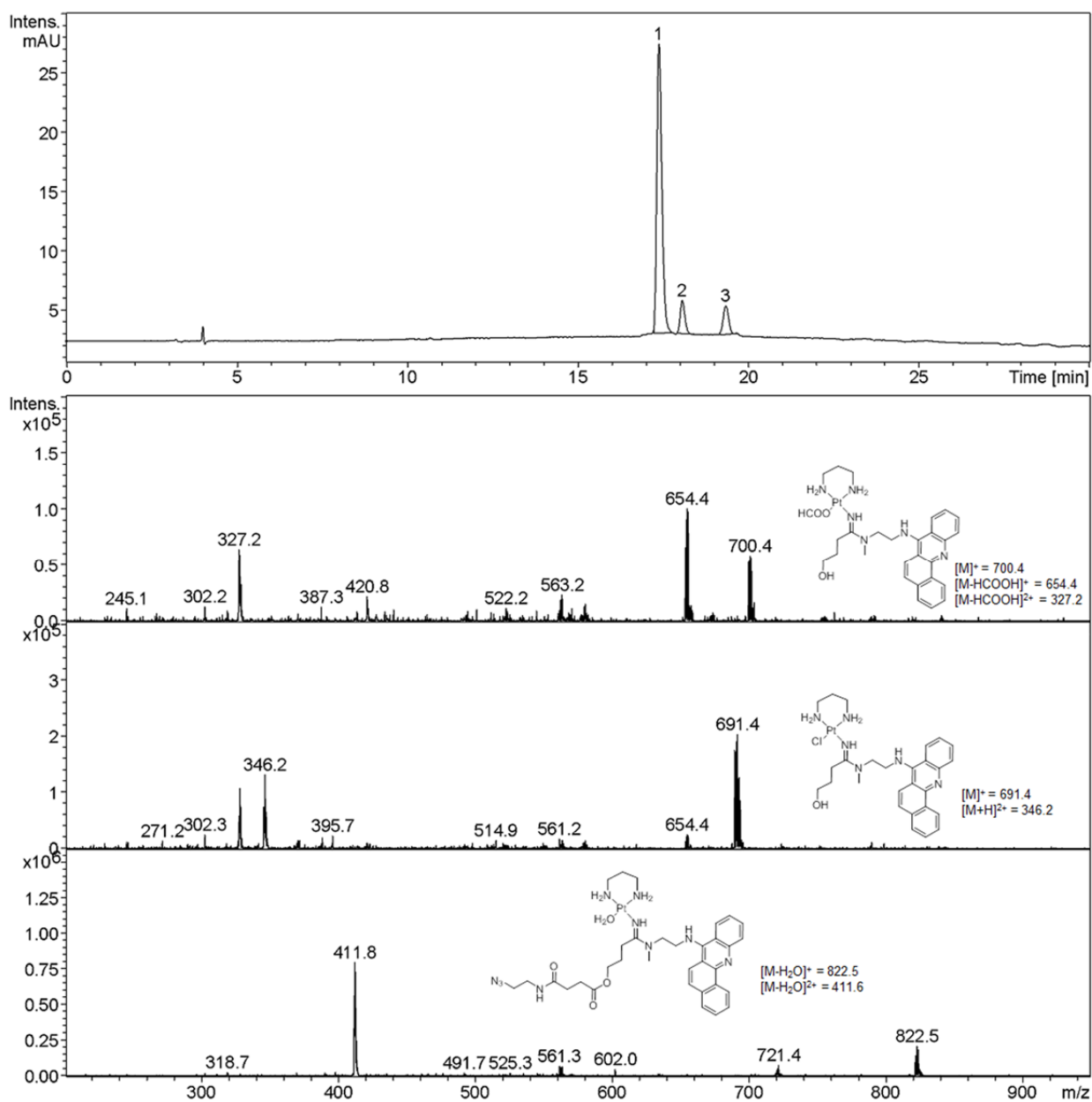


**Figure S30.** LC-ESMS analysis of the mixture of compound **P1-N7** in phosphate buffer (PB, pH 7.4) incubated at 37 °C.

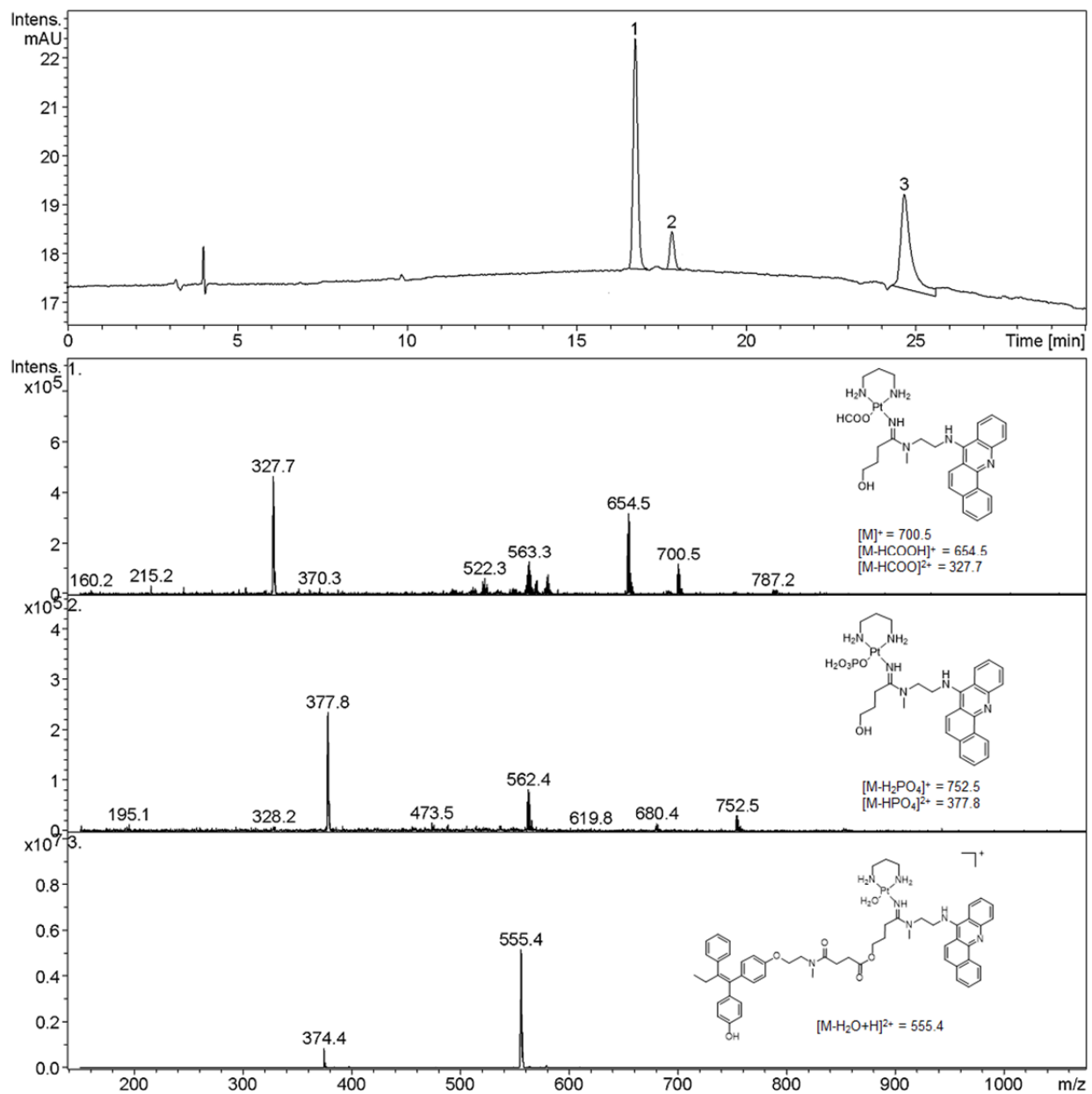




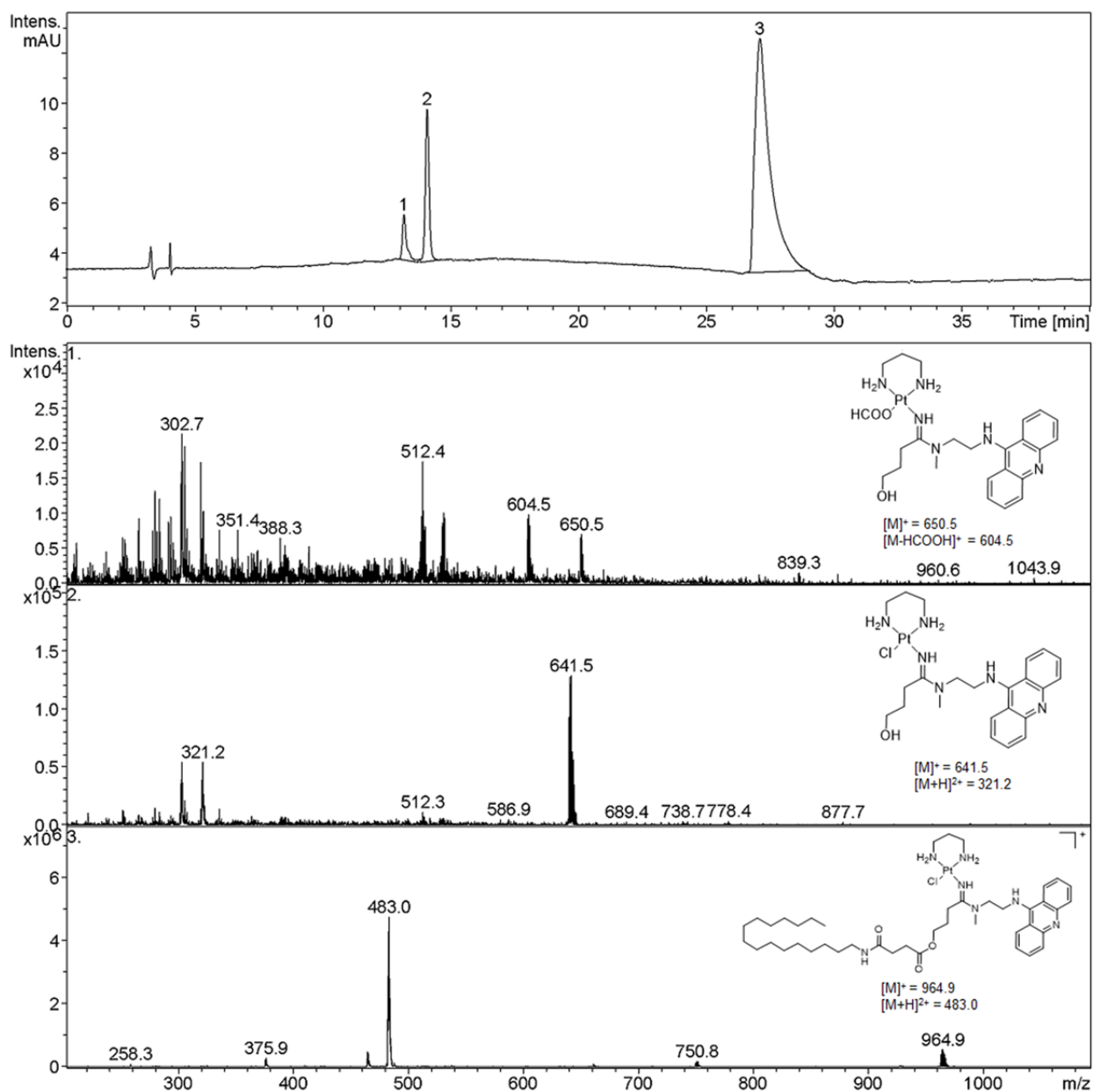
**Figure S31.** LC-ESMS analysis of the mixture of compound **P1-N9** in phosphate buffer (PB, pH 7.4) incubated at 37 °C.



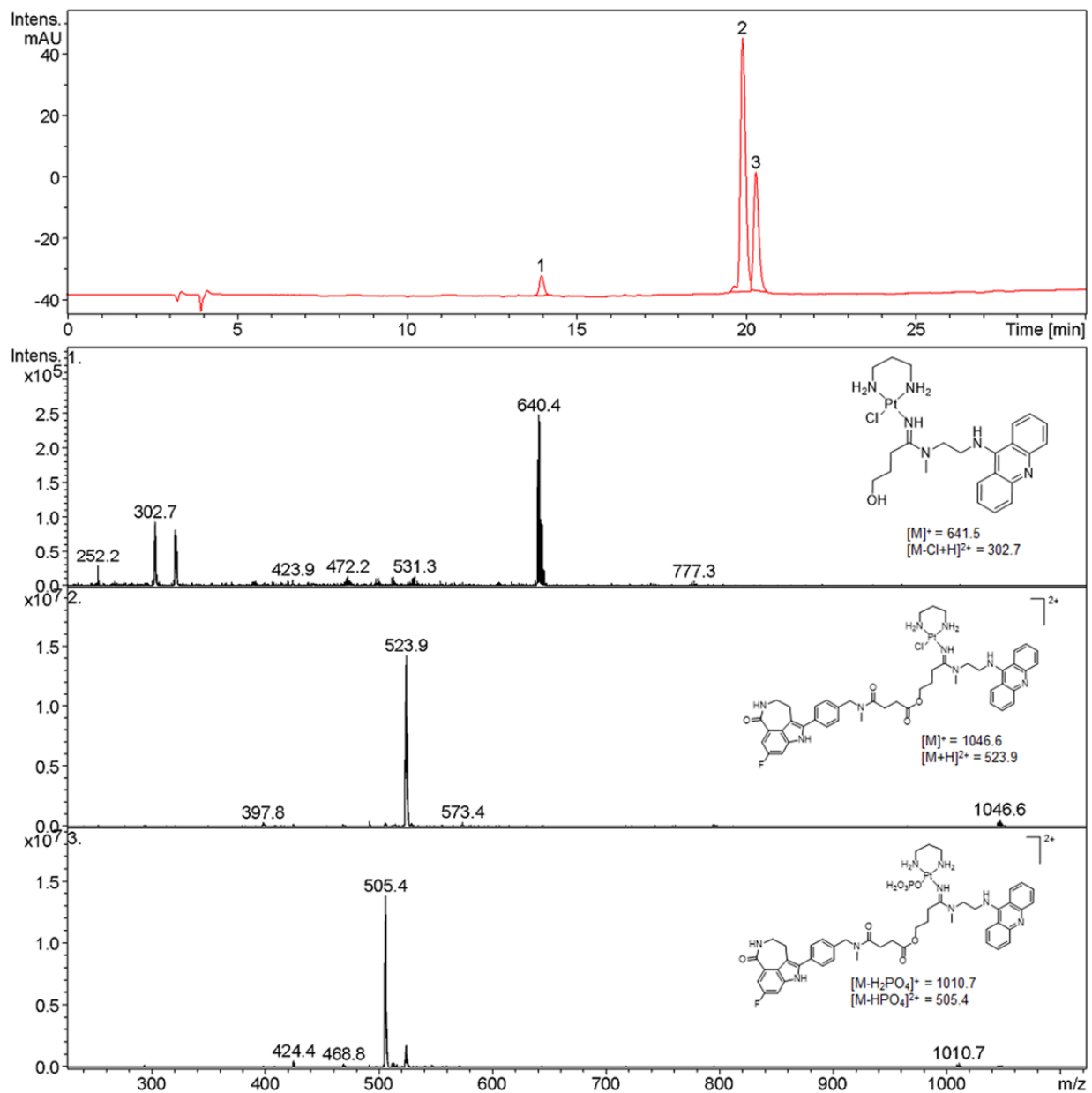
**Figure S32.** LC-ESMS analysis of the mixture of compound **P2-N1** in phosphate buffer (PB, pH 7.4) incubated at 37 °C.



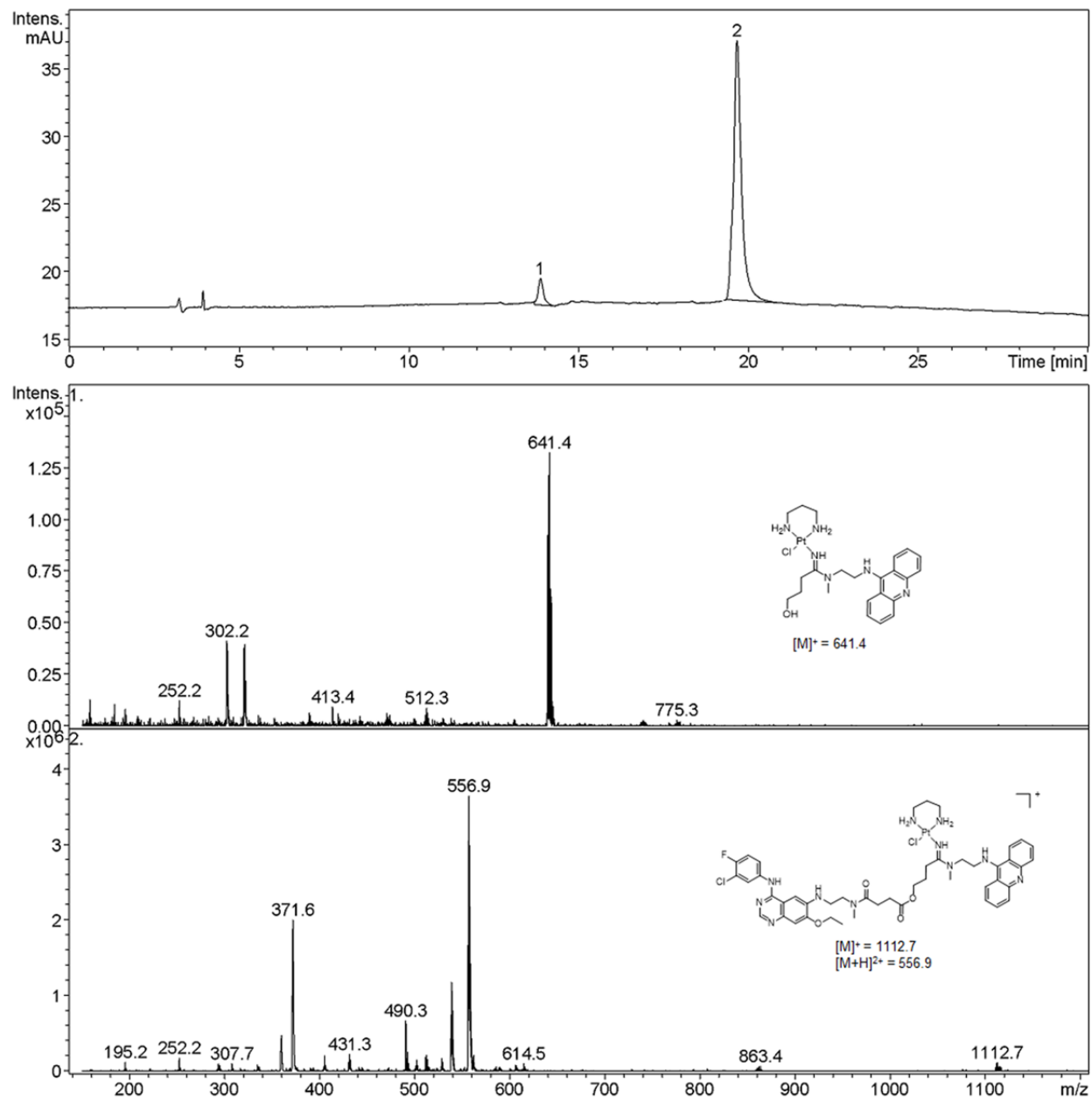
**Figure S33.** LC-ESMS analysis of the mixture of compound **P2-N6** in phosphate buffer (PB, pH 7.4) incubated at 37 °C.



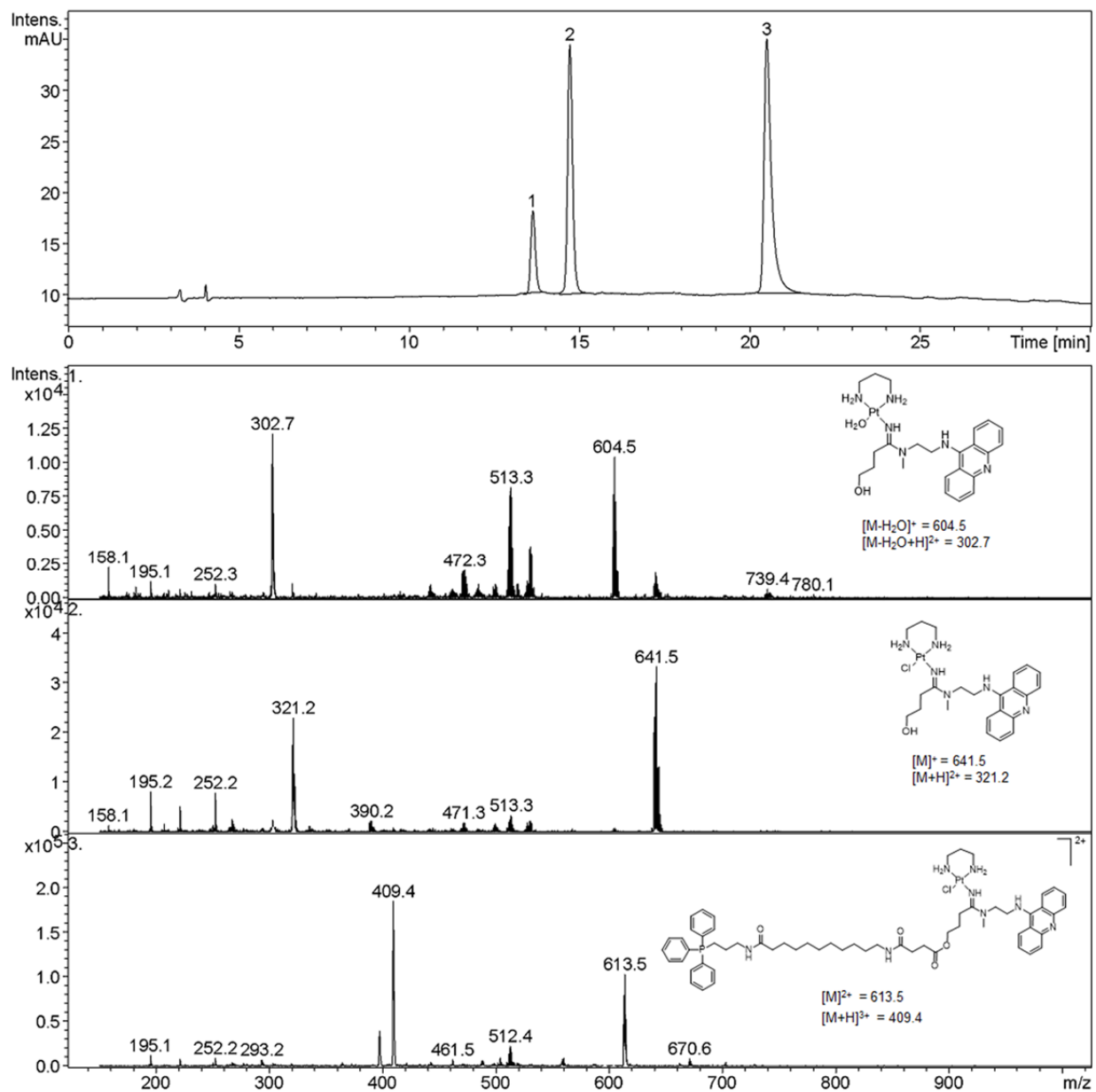
**Figure S34.** LC-ESMS analysis of the mixture of compound **P1-N2** in phosphate buffered saline (PBS, pH 7.4) incubated at 37 °C.



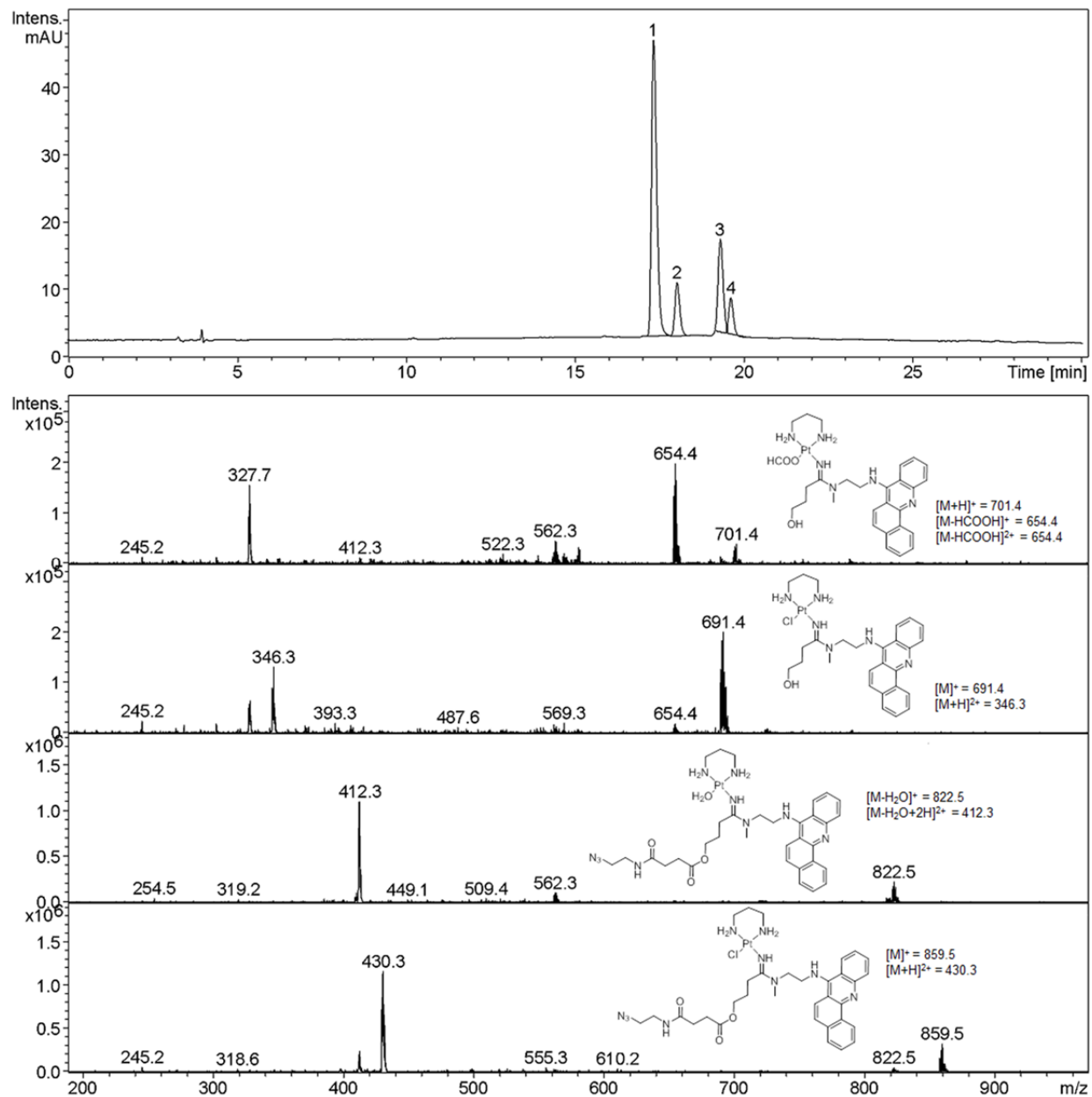
**Figure S35.** LC-ESMS analysis of the mixture of compound **P1-N5** in phosphate buffered saline (PBS, pH 7.4) incubated at 37 °C.



**Figure S36.** LC-ESMS analysis of the mixture of compound **P1-N7** in phosphate buffered saline (PBS, pH 7.4) incubated at 37 °C.

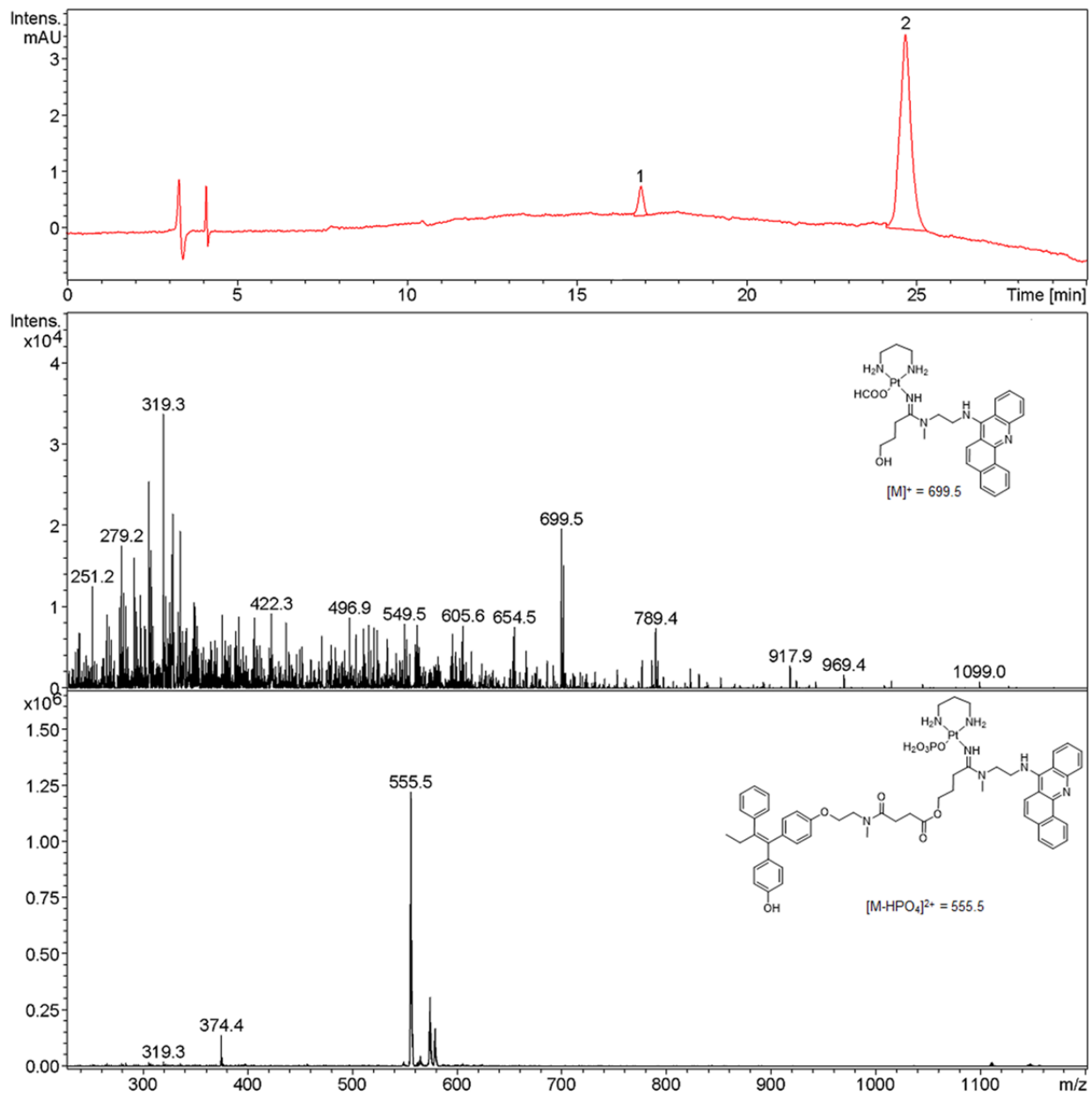


**Figure S37.** LC-ESMS analysis of the mixture of compound **P1-N9** in phosphate buffered saline (PBS, pH 7.4) incubated at 37 °C.



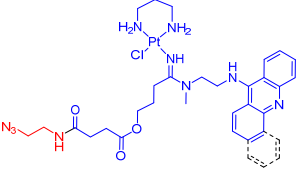
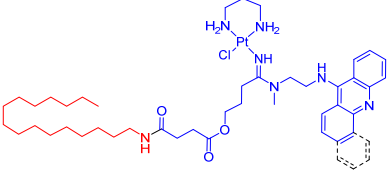
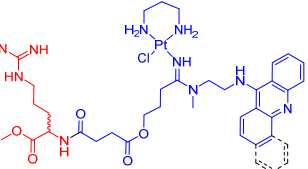
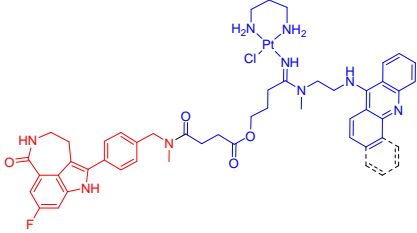
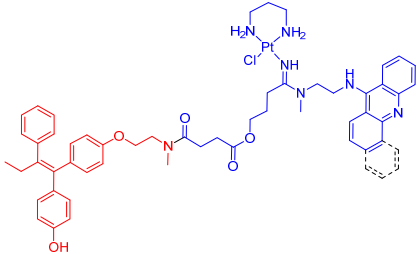
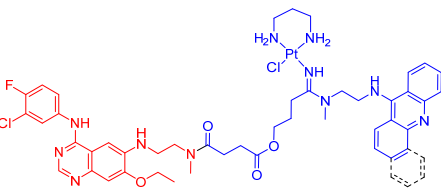
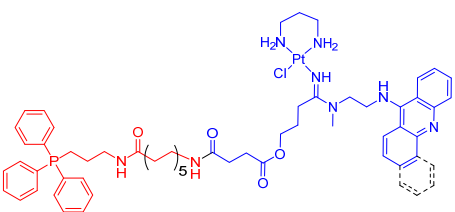
**Figure S38.** LC-ESMS analysis of the mixture of compound **P2-N1** in phosphate buffered saline (PBS, pH 7.4) incubated at 37 °C.





**Figure S39.** LC-ESMS analysis of the mixture of compound **P2-N6** in phosphate buffered saline (PBS, pH 7.4) incubated at 37 °C.

**Table S1.** Conversion Yields and HR-ESMS Results for Conjugates

Compd	Conjugate structures	Conv. (%) $\pm$ S.D. <sup>a</sup>	Calculated [M] <sup>+</sup>	Observed [M] <sup>+</sup>
P1-N1 P2-N1		98.9 $\pm$ 1.4 98.1 $\pm$ 1.7	808.2778 858.2934	808.2786 858.2940
P1-N2 P2-N2		96.6 $\pm$ 1.2 96.9 $\pm$ 2.3	963.4955 1013.5111	963.4954 1013.5125
P1-N4 P2-N4		99.5 $\pm$ 1.9 99.4 $\pm$ 1.1	910.3458 960.3615	910.3466 960.3610
P1-N5 P2-N5		96.4 $\pm$ 2.5 98.9 $\pm$ 1.4	1045.3619 1095.3776	1045.3645 1095.3784
P1-N6 P2-N6		97.1 $\pm$ 2.3 96.7 $\pm$ 0.9	1095.4227 1145.4383	1095.4242 1145.4391
P1-N7 P2-N7		97.8 $\pm$ 1.5 96.4 $\pm$ 2.5	1111.3604 1162.3760	1111.3615 1162.3744
P1-N9 P2-N9		99.8 $\pm$ 0.2 99.7 $\pm$ 0.5	612.7688 637.7766 (2+) <sup>b</sup>	612.7696 637.7769 (2+) <sup>b</sup>

<sup>a</sup> Each reaction was performed in triplicate. The conversion yields were determined from HPLC traces recorded at an acridine-specific wavelength. Reactions with conversion yields lower than 90% are not reported. <sup>b</sup> Singly charged [M]<sup>+</sup> not observed in positive-ion mode. The additional fused ring in the benzofluorene derivatives (**P2**) is shown as dashed bonds.

**Table S2.** Summary of Coupling Reagents Used

Acronym, Common Name	System. Nomenclature	Structure
EDC	<i>N</i> -(3-Dimethylaminopropyl)- <i>N'</i> -ethylcarbodiimide hydrochloride	
DCC	<i>N,N'</i> -Dicyclohexylcarbodiimide	
CDI	1,1'-Carbonyldiimidazole	
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate	
COMU	1-[[1-(Cyano-2-ethoxy-2-oxoethylideneaminoxy)-dimethylamino-morpholinomethylene]] methanaminium hexafluorophosphate	
TSTU	<i>O</i> -( <i>N</i> -Succimidinyl)- <i>N,N,N',N'</i> -tetramethyluronium tetrafluoroborate	
HBTU	<i>O</i> -(Benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate	