Supporting information

Serine- and Threonine-derived Diamine Equivalents for Site-specific Incorporation of Platinum Centers in Peptides, and Anticancer Potential of these Conjugates

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Materials and methods

All amino acids and reagents were purchased from SRL Pvt. Ltd or Spectrochem Pvt. Ltd. and used as received. Potassium tetrachloroplatinate, Calf thymus DNA and Ethidium bromide were purchased from Sigma Aldrich. Solvents were dried following standard procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 400 MHz and 500 MHz instruments. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane, with *J* values in Hertz. ^{13}C NMR spectral data are reported with the solvent peak ($CDCl_3$ at 77.16 ppm, DMSO-D^6 at 39.52ppm) as the internal standard. ^{195}Pt NMR was recorded on 500 MHz instrument and chemical shifts were reported relative to K_2PtCl_4 (δ = -1617ppm) as external standard. High-resolution mass spectra (HRMS) were recorded on a Waters Q-Tof *micro*™ spectrometer with lock spray source. Infrared spectra were recorded using a Nicolet 6700 FT-IR spectrophotometer.

General experimental procedure:

b) Peptide coupling using EDC: A mixture of the acid (1 eq), EDC (1.5 eq), HOBt (1.5 eq) and triethylamine (3 eq) in CH_2Cl_2 was stirred at 0 ºC under an inert atmosphere for about 15 min. Solutions of free-amines (1.2 eq) in CH_2Cl_2 were added slowly to the reaction mixture and the mixture left stirred until the starting materials were completely consumed as per TLC. After completion of the reaction, the mixture was sequentially washed with aqueous saturated $NaHCO_3$ and 5% aqueous HCl. The dichloromethane layer was dried, evaporated under reduced pressure and the crude material was purified by column chromatography (ethyl acetate/hexane) to get the peptides in good to excellent yields.

c) General procedure for the hydrolysis of methyl ester: Methyl ester of peptide/amino acid was dissolved in a mixture of THF:water (2:1) and LiOH (1.5 equiv.) was added and stirred until starting material was consumed. The organic solvent (THF) was evaporated, diluted with

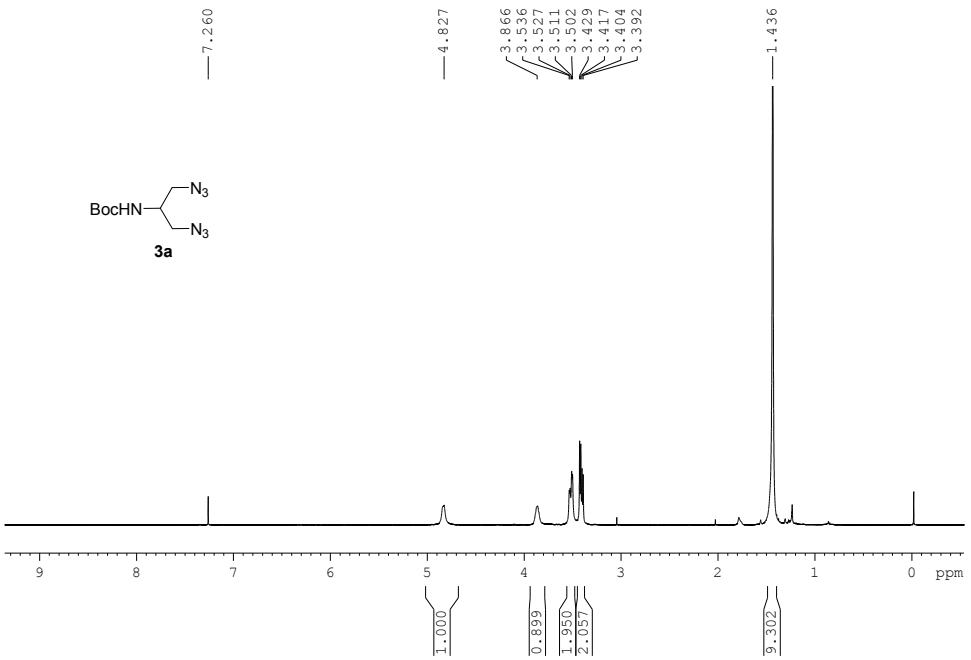
water and neutralized with dil.HCl. The product was extracted with Ethylacetate, dried (Na_2SO_4) and evaporated to get the acid that was directly used for further transformation.

d) General procedure for Pd/C, H_2 reduction of diazides: The diazide was dissolved in methanol in an RB flask and 10% Pd/C (30mg/100mg) was added to it. The reaction mixture was stirred for 6h-24h under a positive pressure of hydrogen (balloon). It was filtered through celite, methanol was evaporated under reduced pressure, and washed with hexane to get the product as a semi-solid which was used directly for platinum complexation.

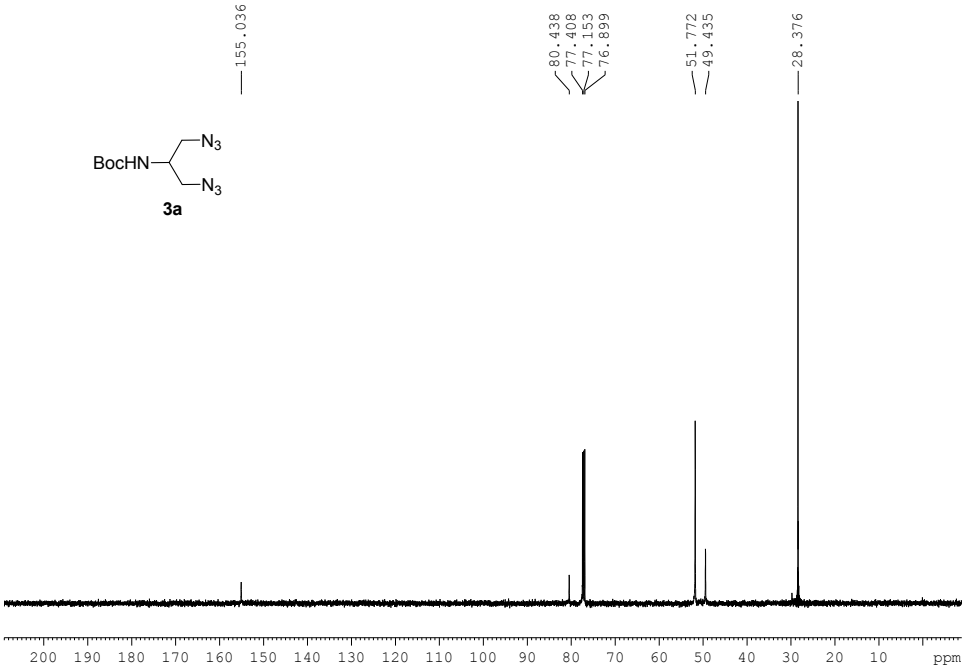
e) General procedure for amidation of esters: The Boc protected methyl ester of peptides were dissolved in a mixture of methanol and 25% aqueous ammonia (1:2), and stirred until starting material was consumed. Methanol was evaporated under reduced pressure, water was added and it was extracted with ethyl acetate (3 x 20 mL). Ethyl acetate was dried (Na_2SO_4) and evaporated to get the crude product which was purified by column chromatography (ethylacetate/hexane).

f) Synthesis of orthogonally protected Lysine (NH_2-(ε-Boc)Lys-OMe: Orthogonally protected lysine was prepared according to reported procedure (*Biomacromolecules*, **2016**, 17, 2399 and *Tetrahedron Letters*, **2006**, 47, 5159). Initially, L-lysine mono hydrochloride (2.6 mmol) was dissolved in aqueous 2M $NaHCO_3$ (7.8 mmol) and $CuSO_4.5H_2O$ (1.3 mmol) followed by a solution of diter-butyldicarbonate (3.4 mmol) in acetone were added to it. Stirring was continued for 24 hours, methanol (15 mL) was added and stirring was continued for 12 h. The blue coloured copper–lysine complex slurry was filtered and dried using vacuum pump. This solid was suspended in water, sodium sulphide (1.3 mmol) was added, and stirred for 15 minutes. The black precipitate of CuS was filtered off and the filtrate was treated with Na_2CO_3 and Benzyloxycarbonylchloride (CBzCl). The reaction was continued for 24 h after which it was neutralised with citric acid, extracted with Ethyl acetate, dried (Na_2SO_4) and evaporated to get the crude product which was purified by column chromatography using Ethyl acetate/Hexane. The pure acid obtained was esterified by treatment with MeI in DMF/K_2CO_3

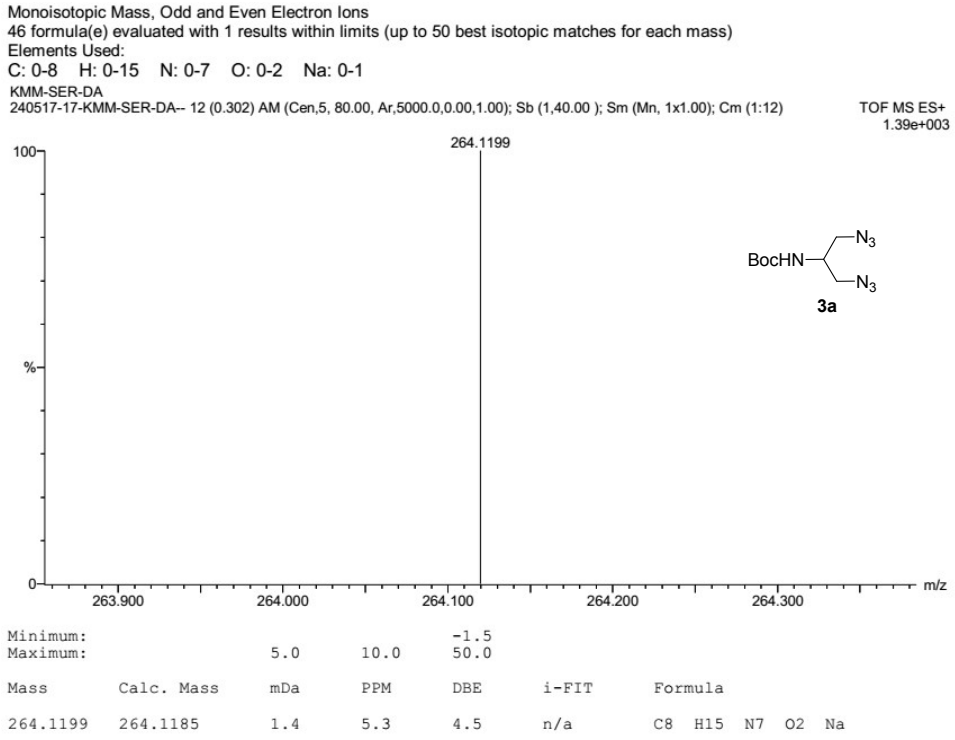
for 24 h. Solvent was evaporated, washed with water and the product was extracted using ethylacetate. This completely protected Lysine (N^{α}-Cbz-N^{ε}-Boc-Lysine-OMe) was treated with 10% Pd/C under hydrogen atmosphere to get the title compound.



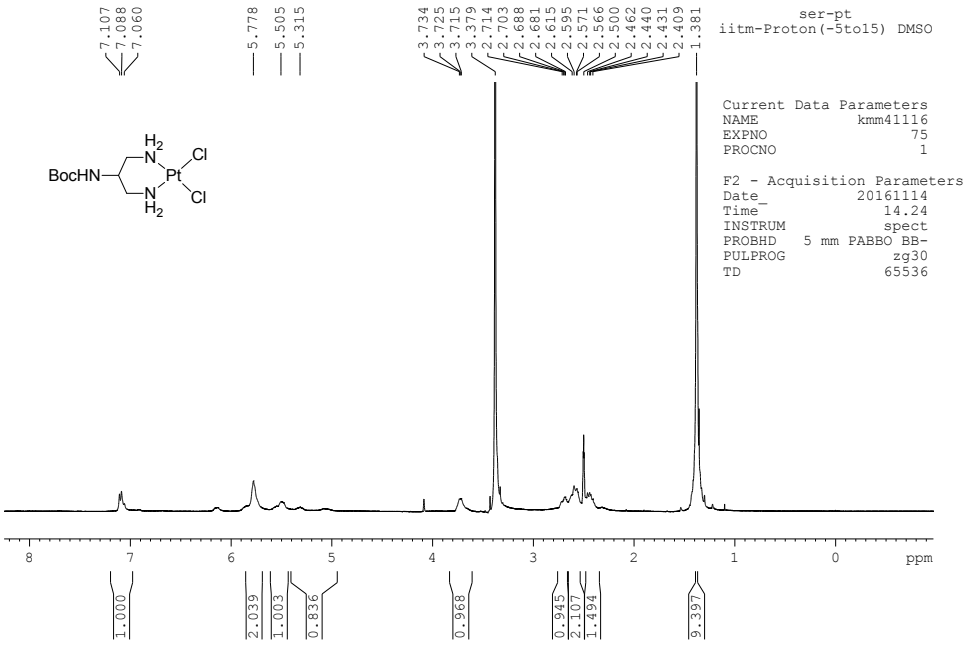
S1: ^{1}H NMR of **3a** (500MHz, $CDCl_3$).



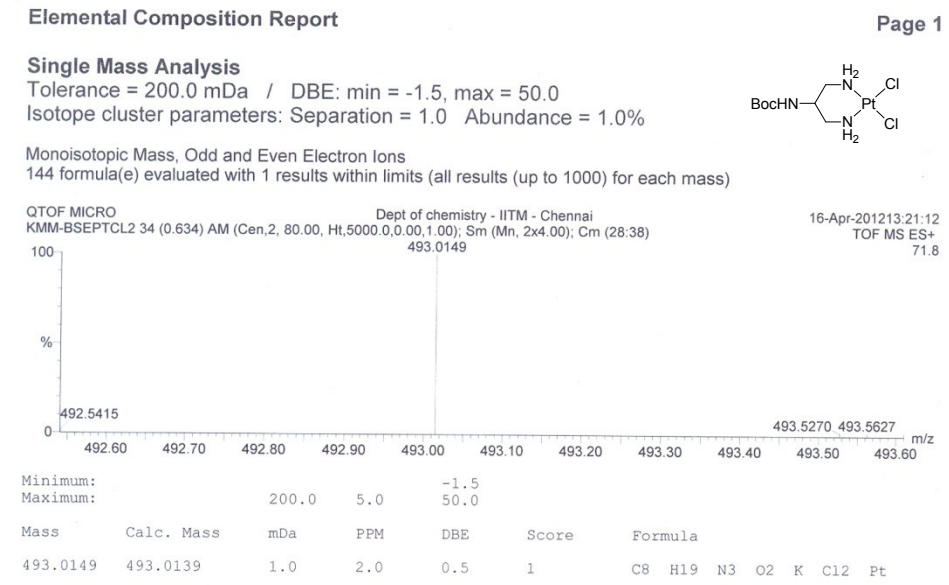
S2: ^{13}C NMR of **3a** (125 MHz, $CDCl_3$).



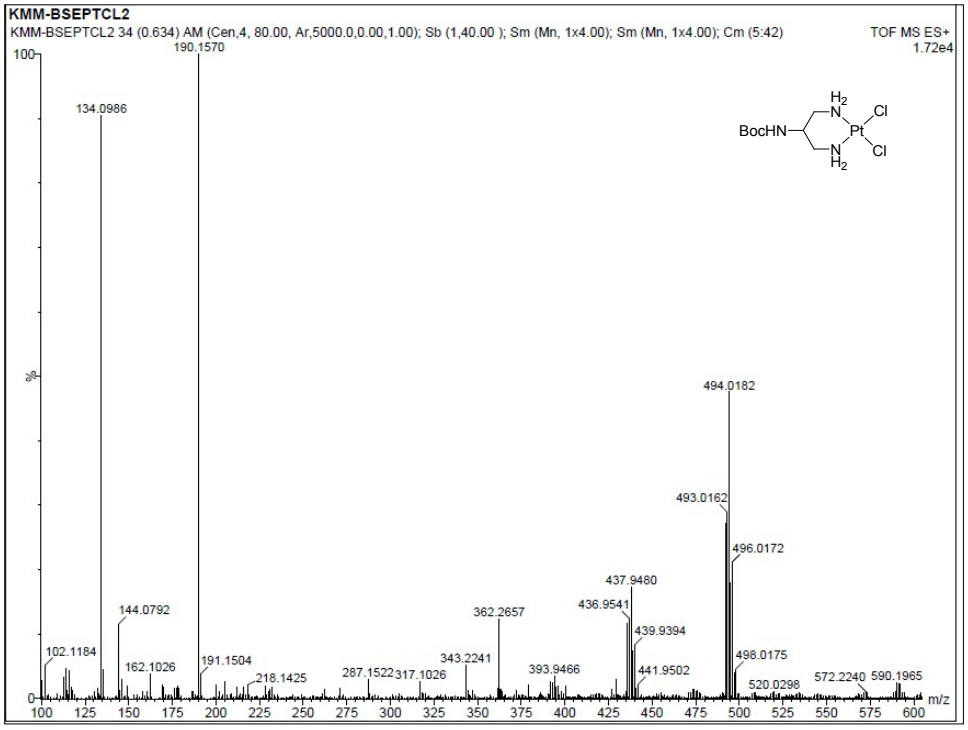
S3: HRMS of **3a**.



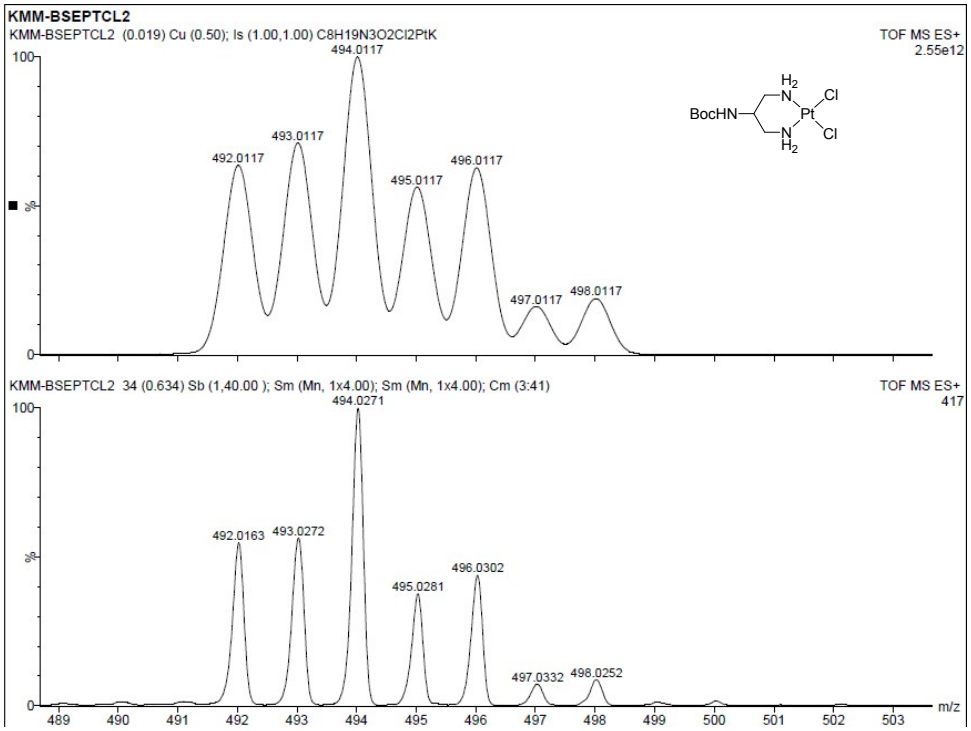
S4: ^{1}H NMR of **4a** (500MHz, DMSO-d_6).



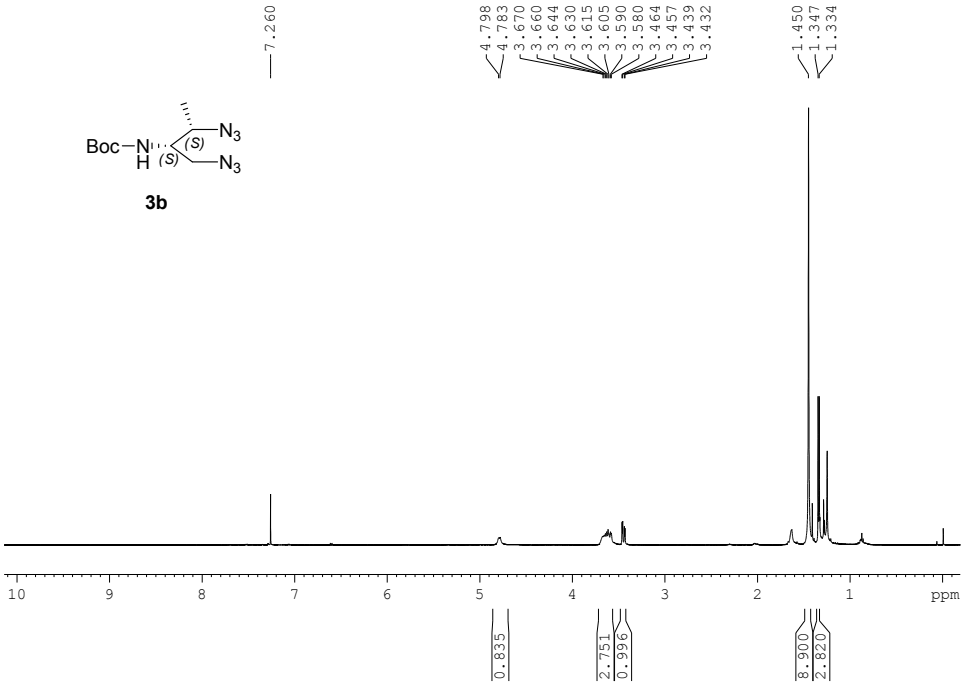
S5: HRMS of **4a**.



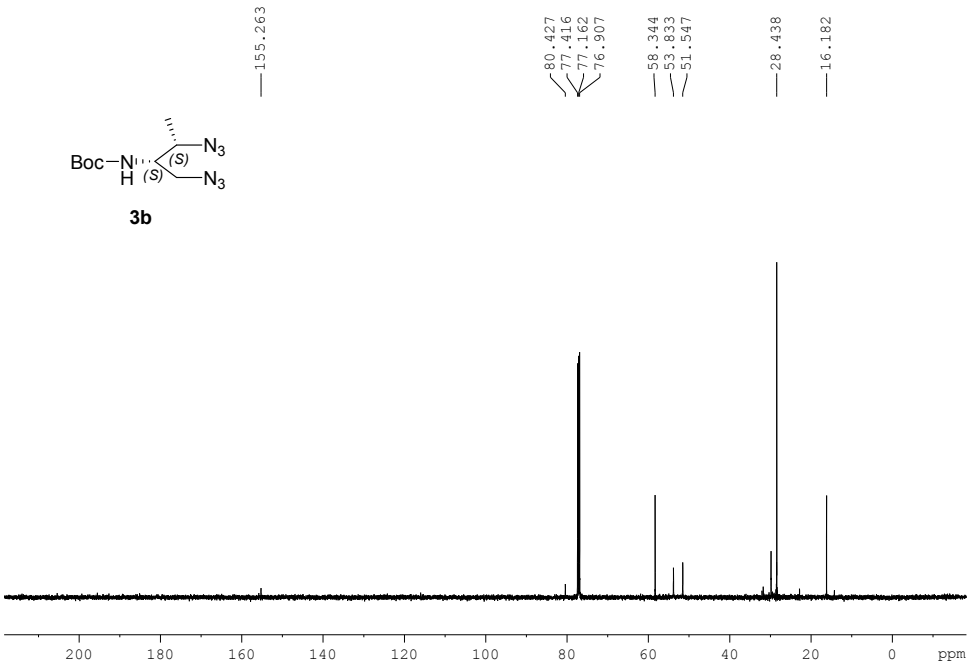
S6: MS spectrum of **4a**.



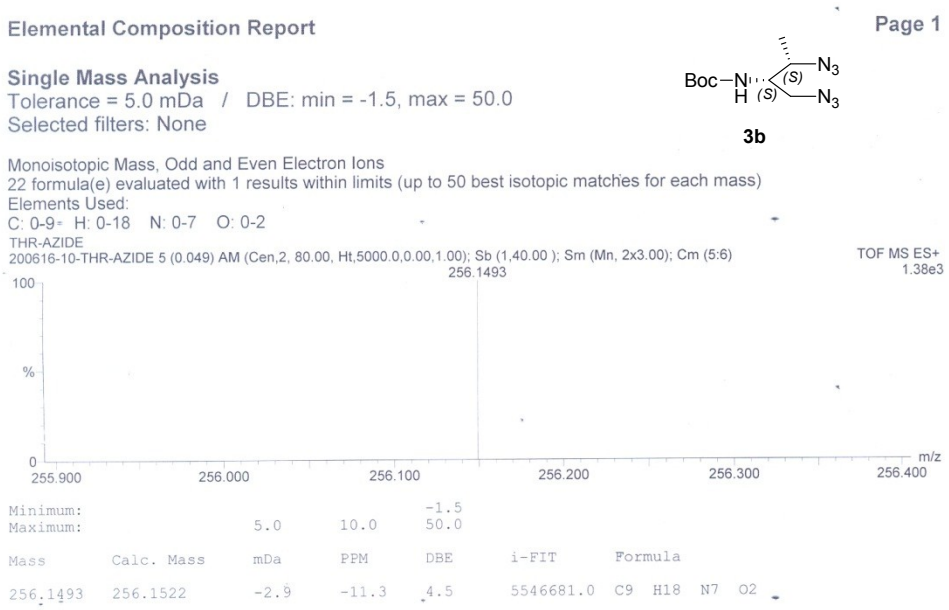
S7: isotopic pattern theoretical (above) and experimental (below) corresponding to $[M+K]^+$ of **4a**.



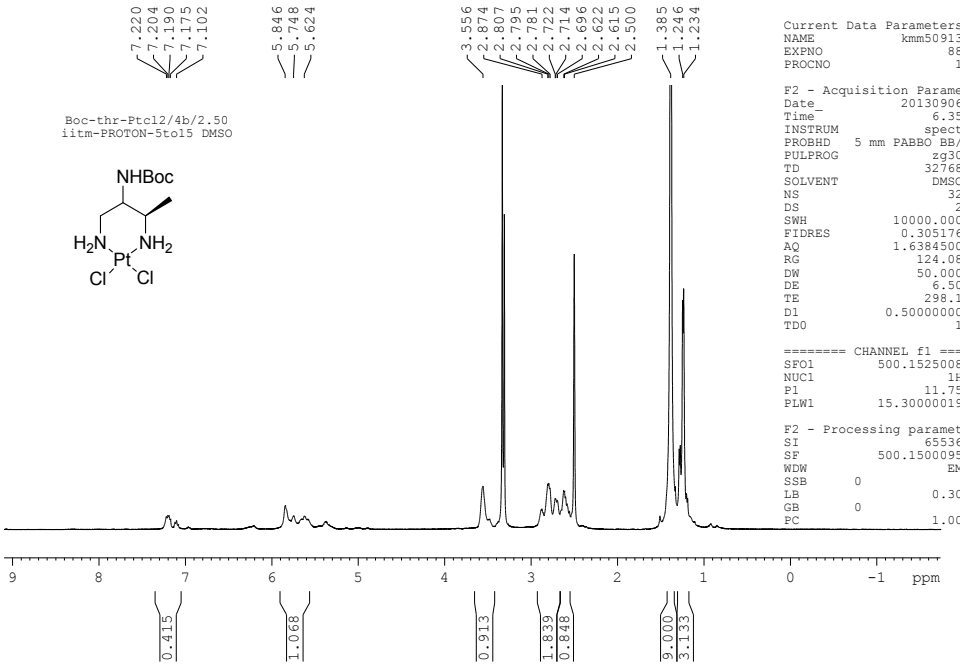
S8: ^{1}H NMR of **3b** (500MHz, $CDCl_3$).



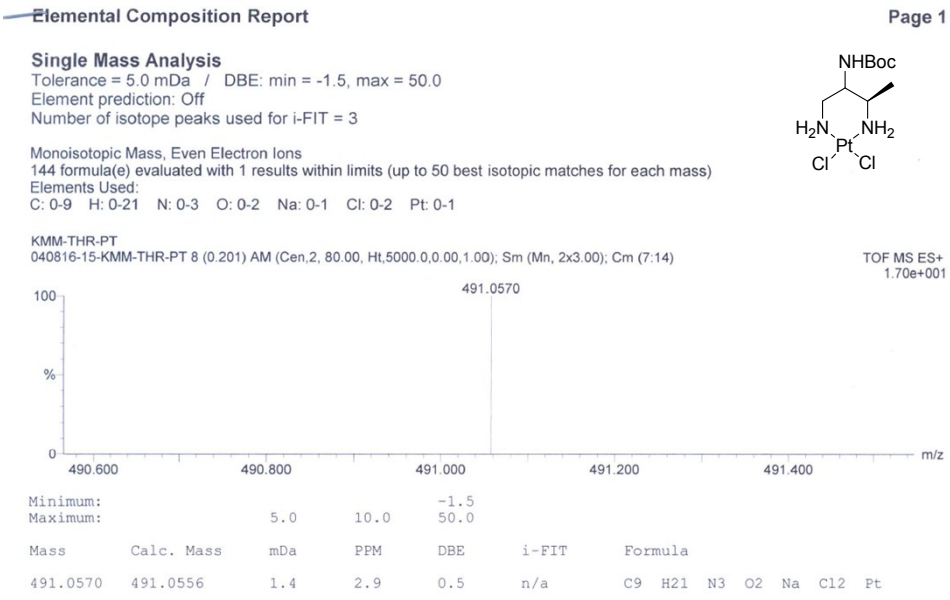
S9: ^{13}C NMR of **3b** (125MHz, $CDCl_3$).



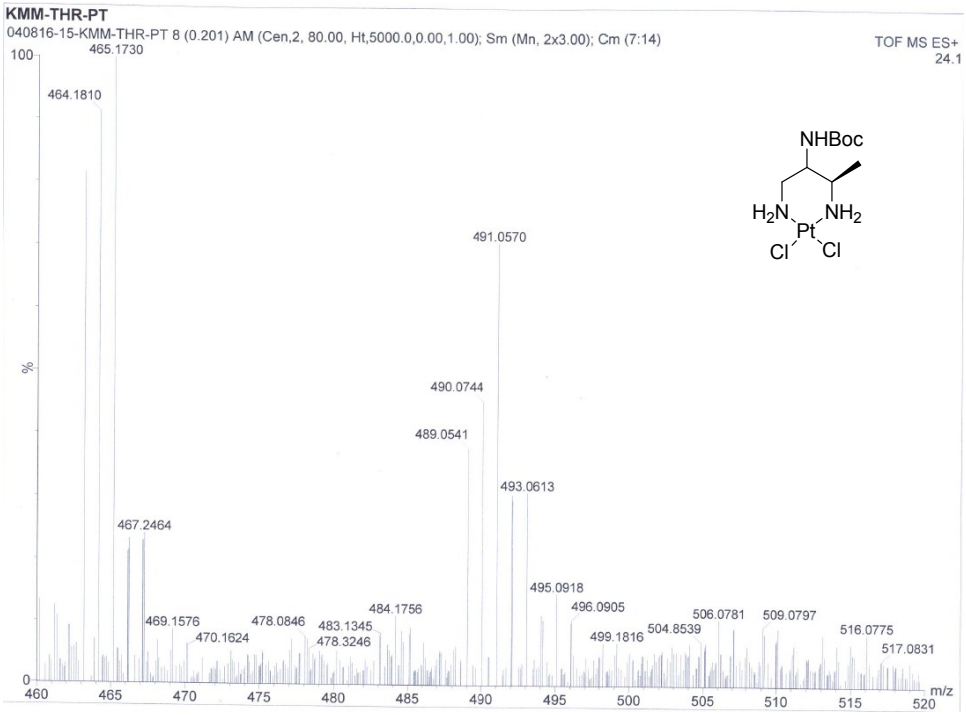
S10: HRMS of **3b**.



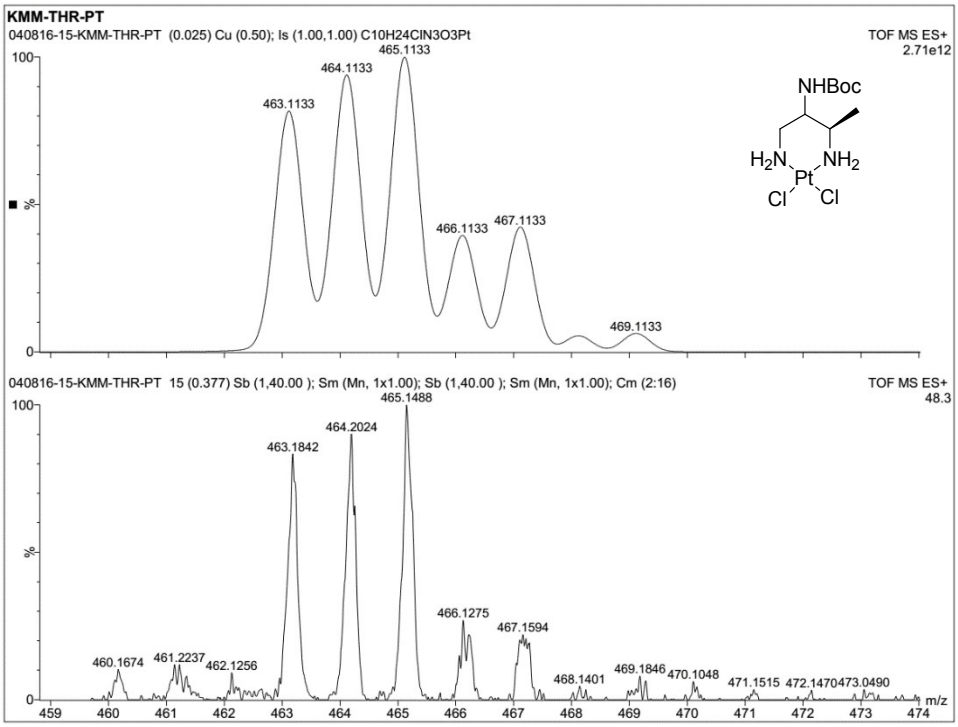
S11: ^{1}H NMR of **4b**.



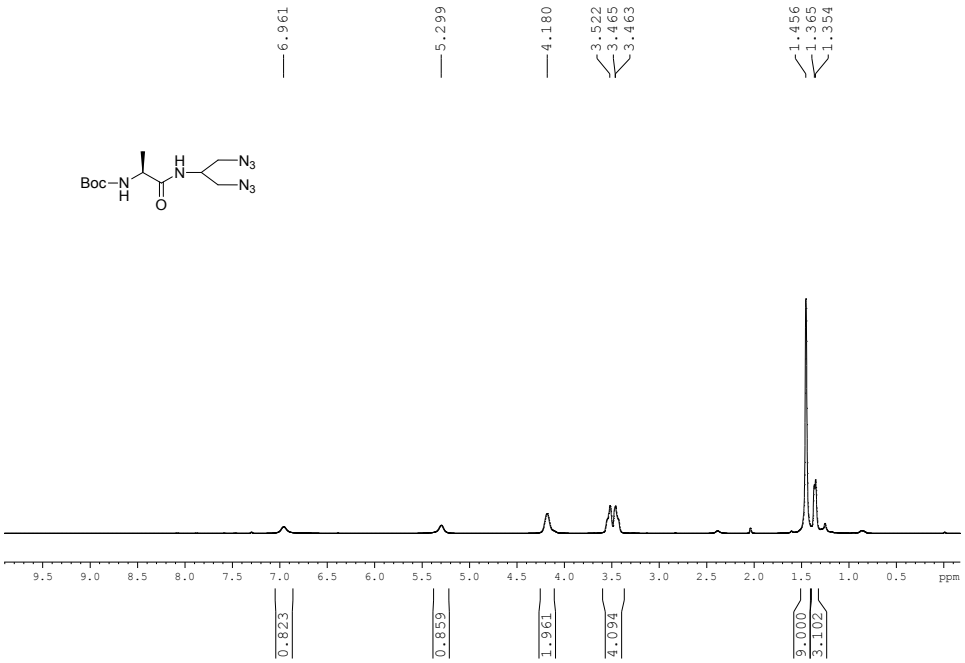
S12: HRMS of **4b**.



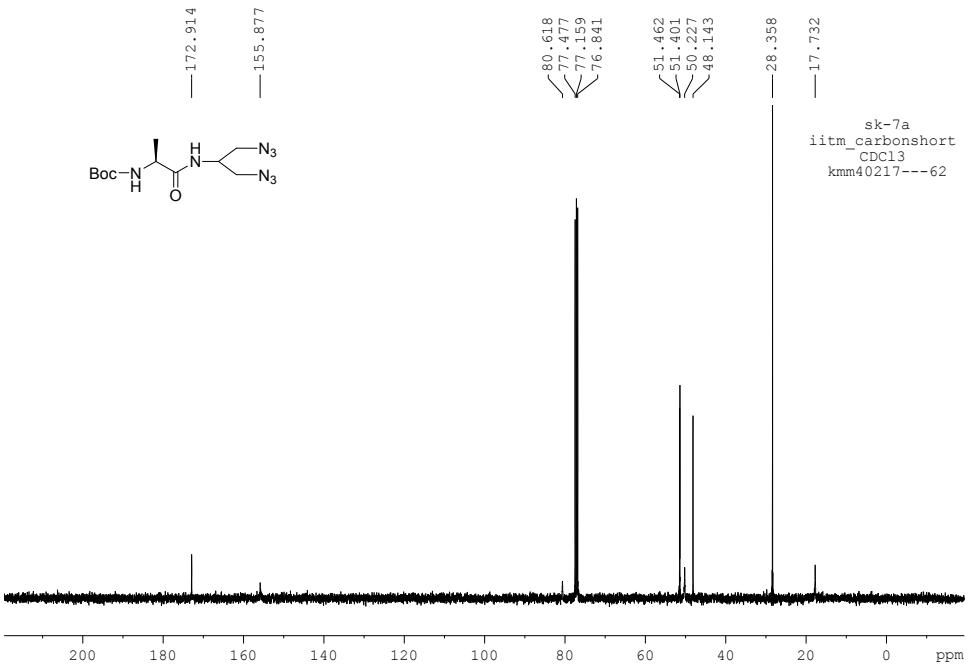
S13: MS spectrum **4b**.



S14: isotopic pattern corresponding to $[M\text{-}Cl+CH_3OH]^+$ of **4b** theoretical (above) and experimental (below).



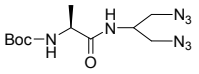
S15: ^{1}H NMR **of Diazide 6a** (precursor of **7a**; 500 MHz, $CDCl_3$).



S16: ^{13}C DEPT of Diazide **6a** (precursor of **7a**; 125 MHz, $CDCl_3$).

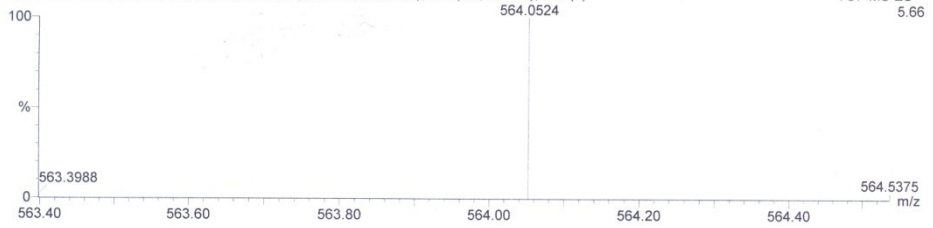
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 200.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%



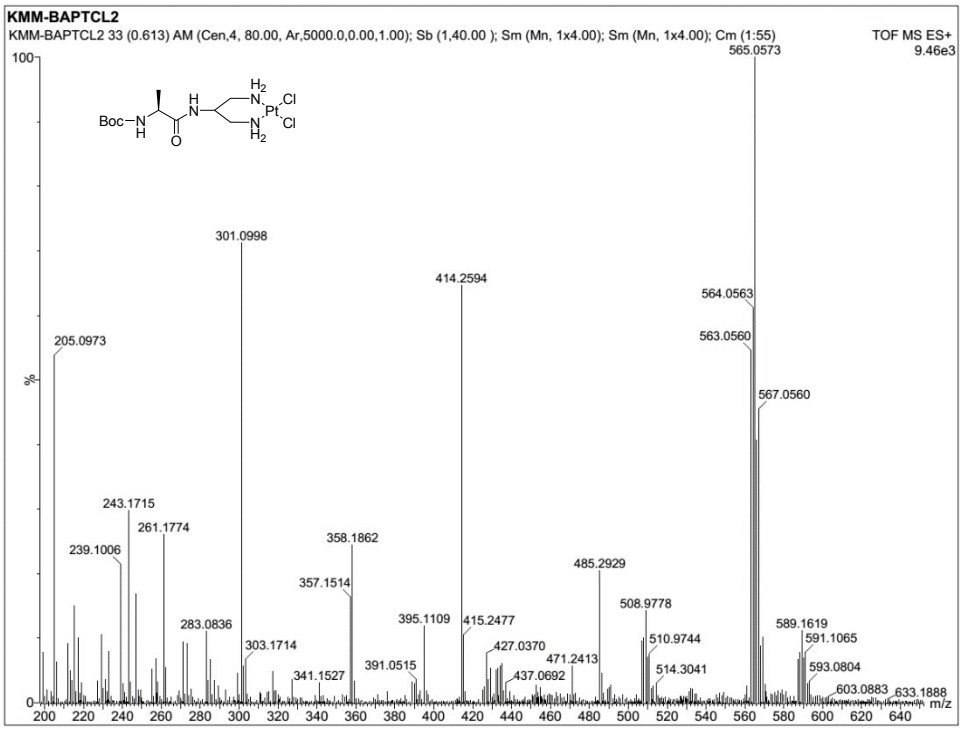
Monoisotopic Mass, Odd and Even Electron Ions
240 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

QTOF MICRO Dept of chemistry - IITM - Chennai 16-Apr-201212:22:30
KMM-BAPTCL2 1 (0.019) AM (Cen,2, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x4.00); Cm (1) TOF MS ES+
564.0524 5.66

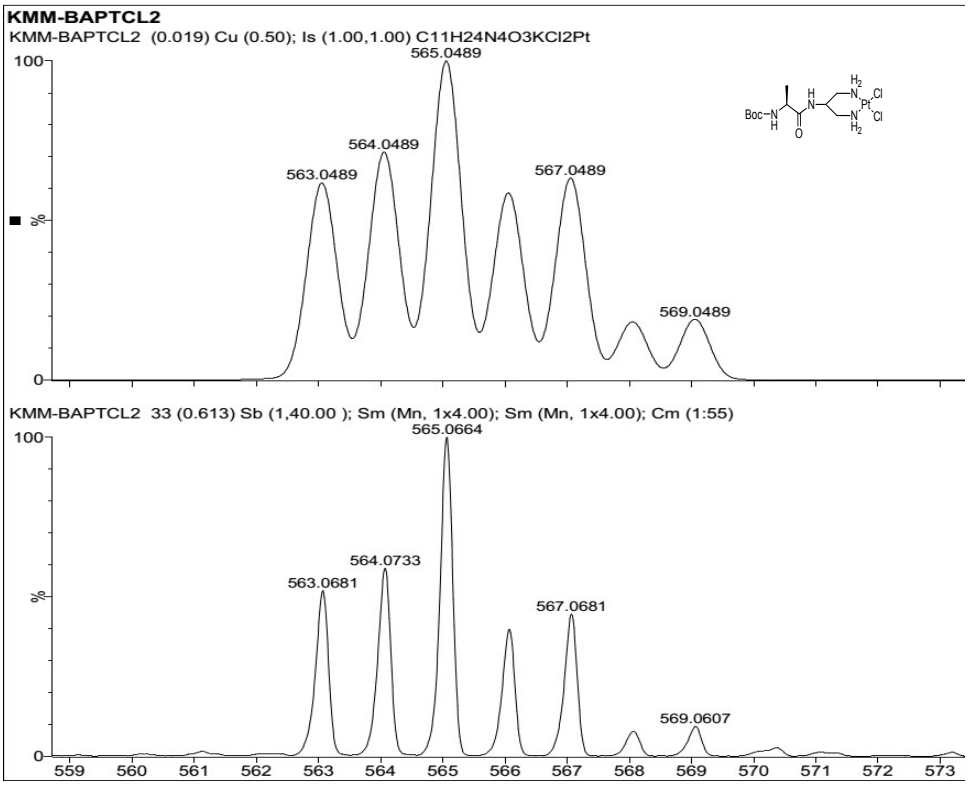


Minimum:				-1.5		
Maximum:		200.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
564.0524	564.0510	1.4	2.5	1.5	1	C11 H24 N4 O3 K Cl2 Pt

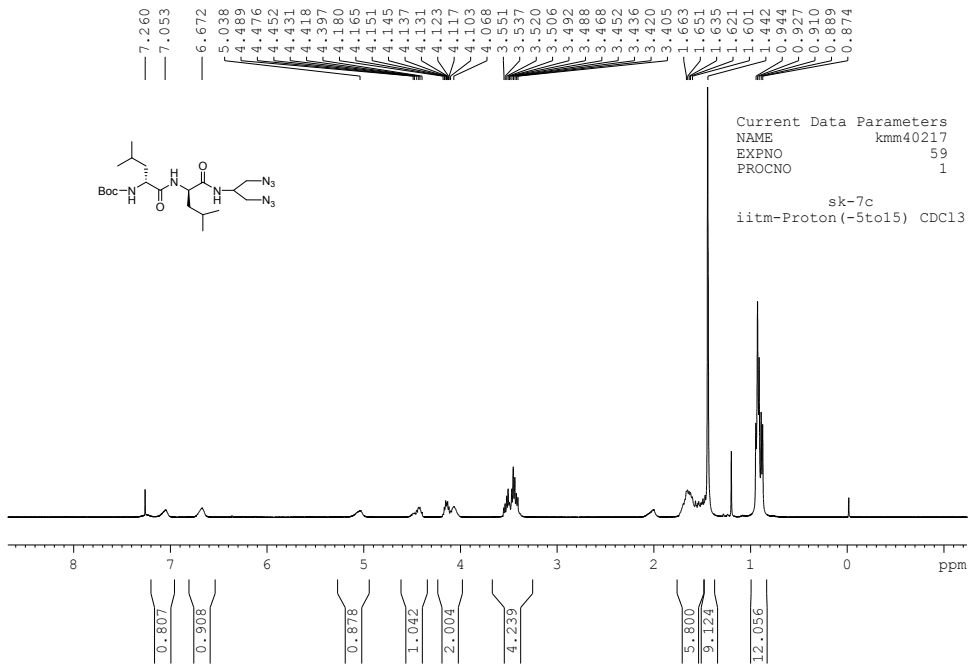
S17: HRMS of **7a**.



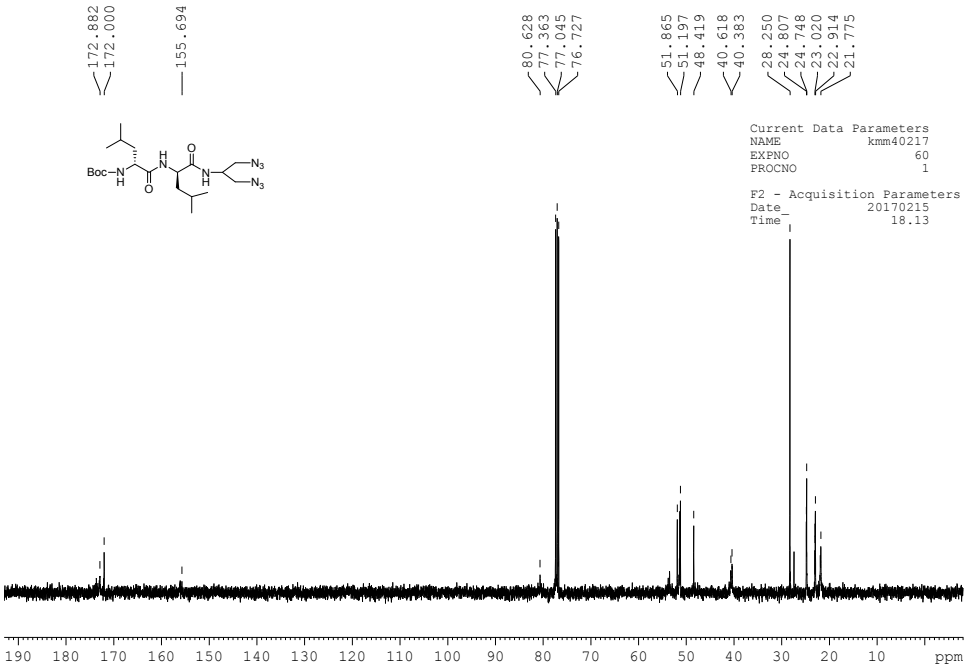
S18: MS spectrum of **7a**.



S19: isotopic pattern theoretical (above) and experimental (below) corresponding to $[M+K]^+$ of **7a**.



S20: ^{1}H NMR of Diazide **6b** (precursor of **7b**; 500MHz, $CDCl_3$).



S21: ^{13}C NMR of Diazide **6b** (precursor of **7b**; 125 MHz, $CDCl_3$).

Single Mass Analysis
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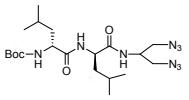
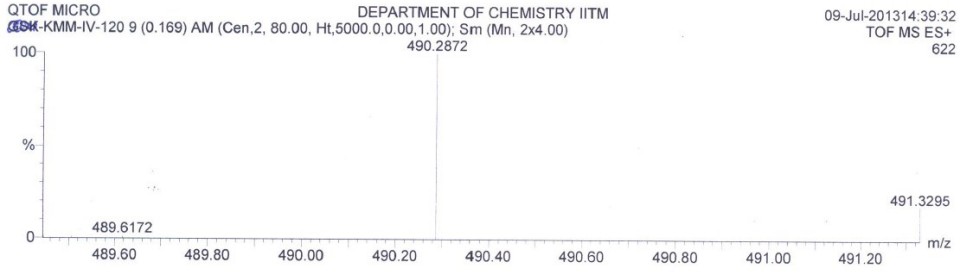
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%



Monoisotopic Mass, Odd and Even Electron Ions
97 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)



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Maximum:		200.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
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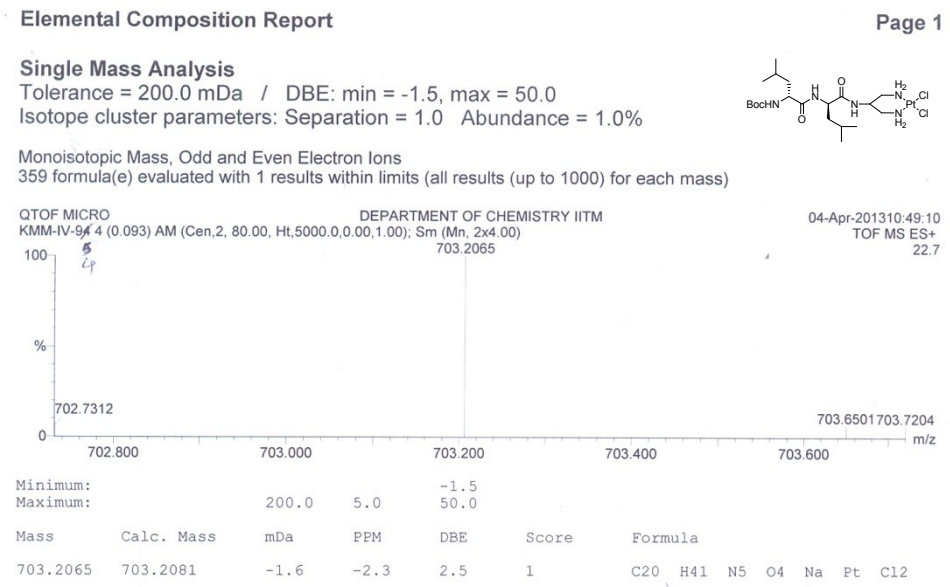
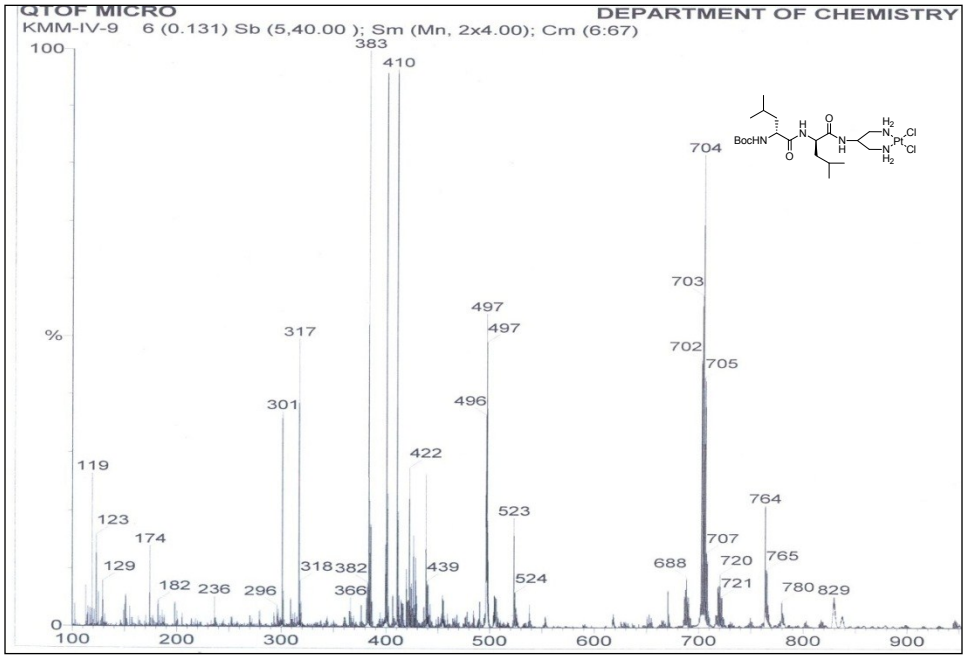
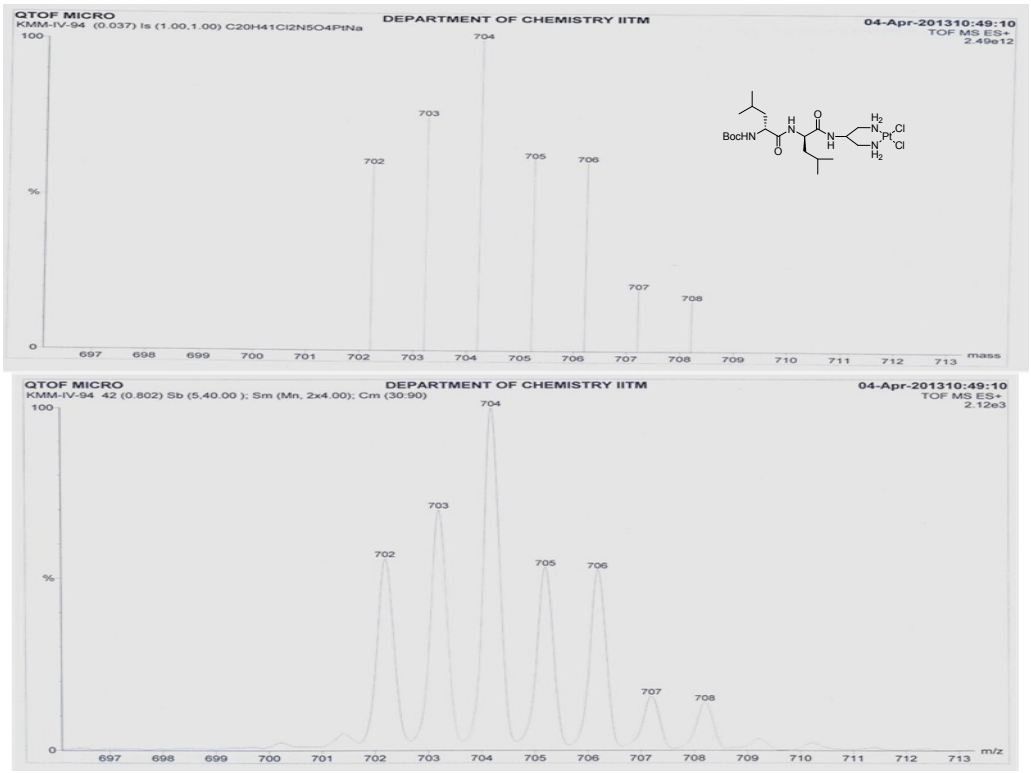
S22 : HRMS of Diazide **6b** (precursor of **7b**).



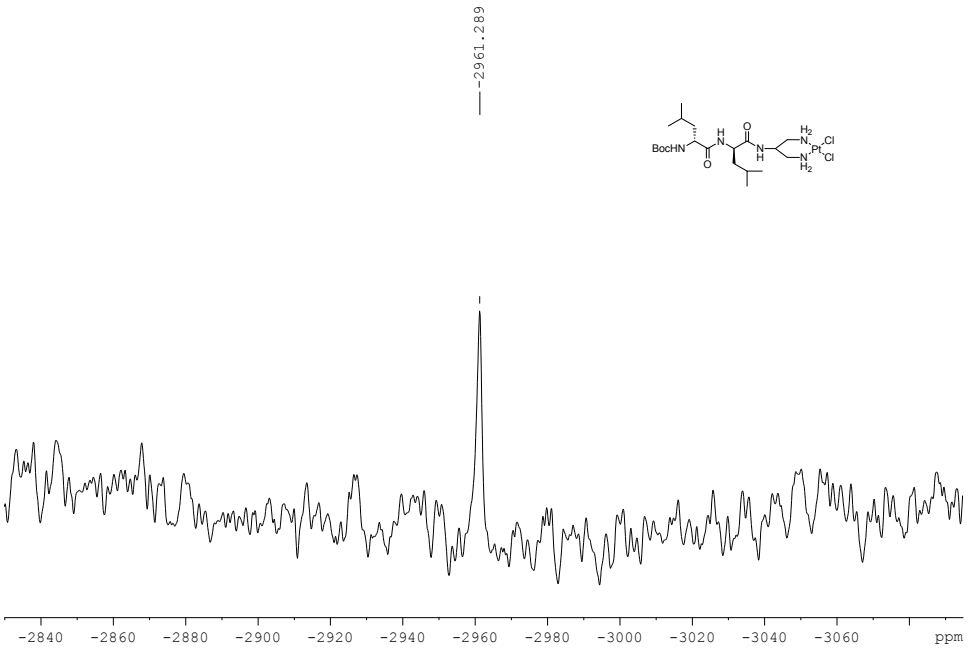
S23: HRMS of **7b**.



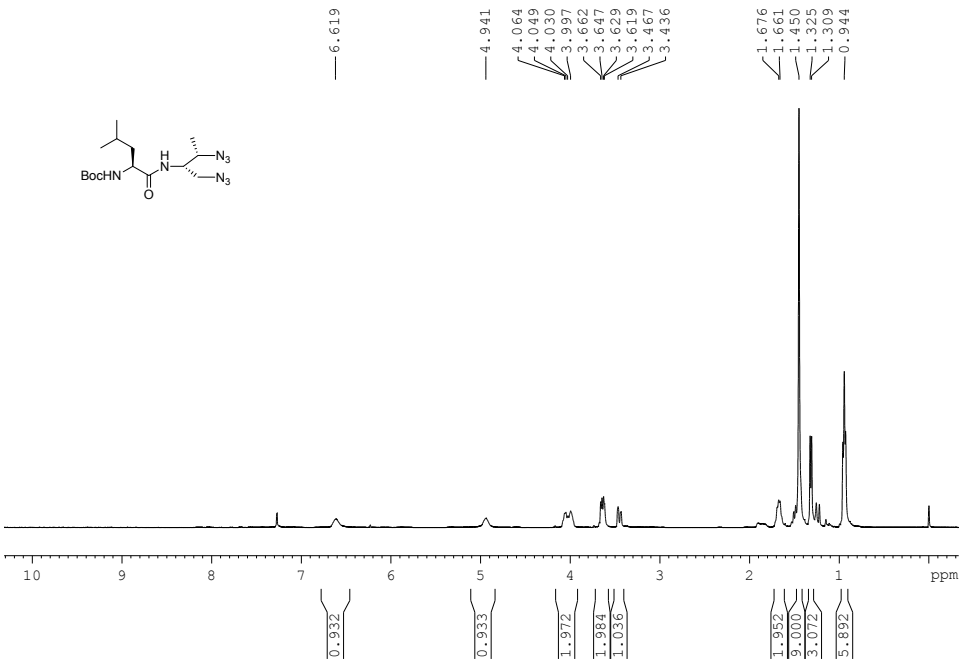
S24: ESI MS spectrum of **7b**.



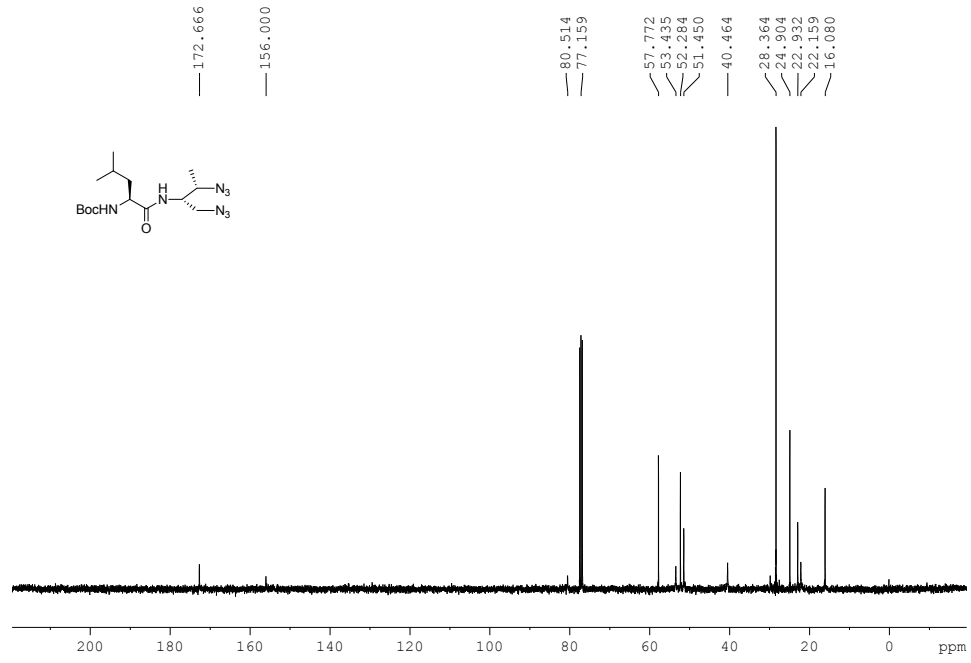
S25: isotopic pattern theoretical (above) and experimental (below) corresponding to $[M+K]^+$ of **7b**.



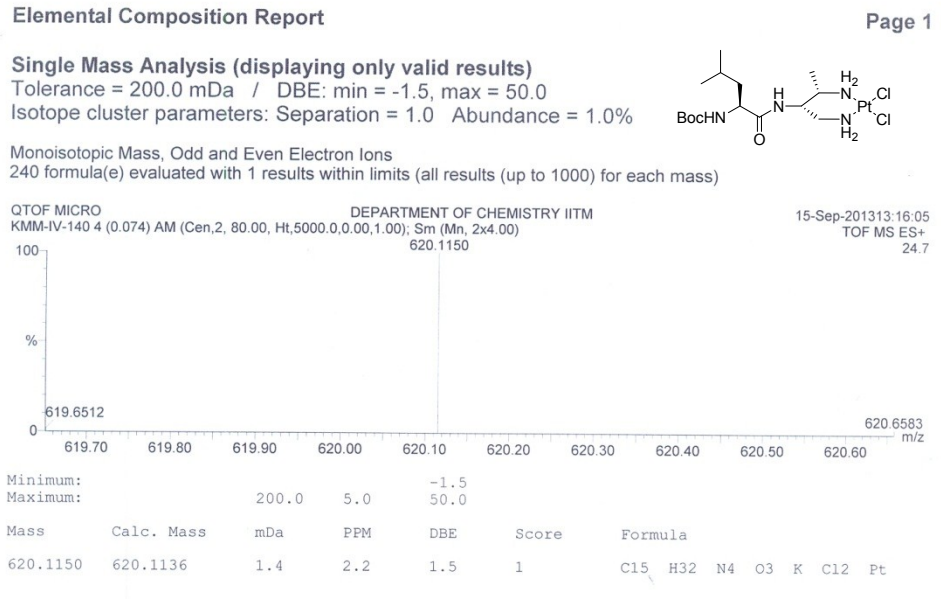
S26: ^{195}Pt NMR of **7b**. (107 MHz, DMSO-d_6)



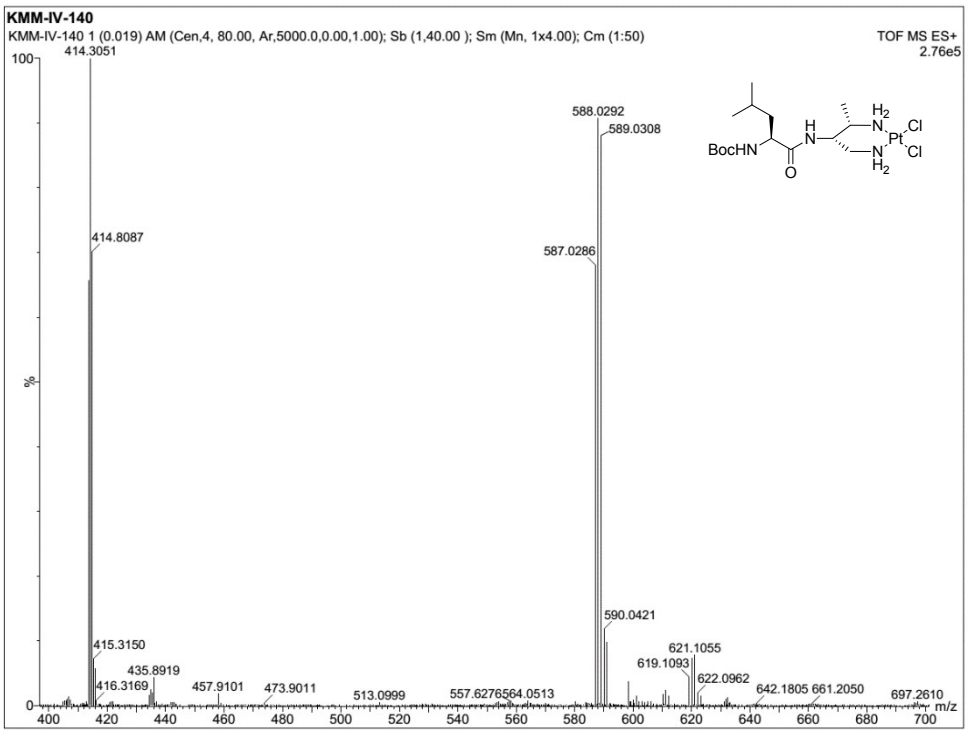
S27: ^{1}H NMR of Diazide **6c** (precursor of **7c**; 500 MHz, $CDCl_3$).



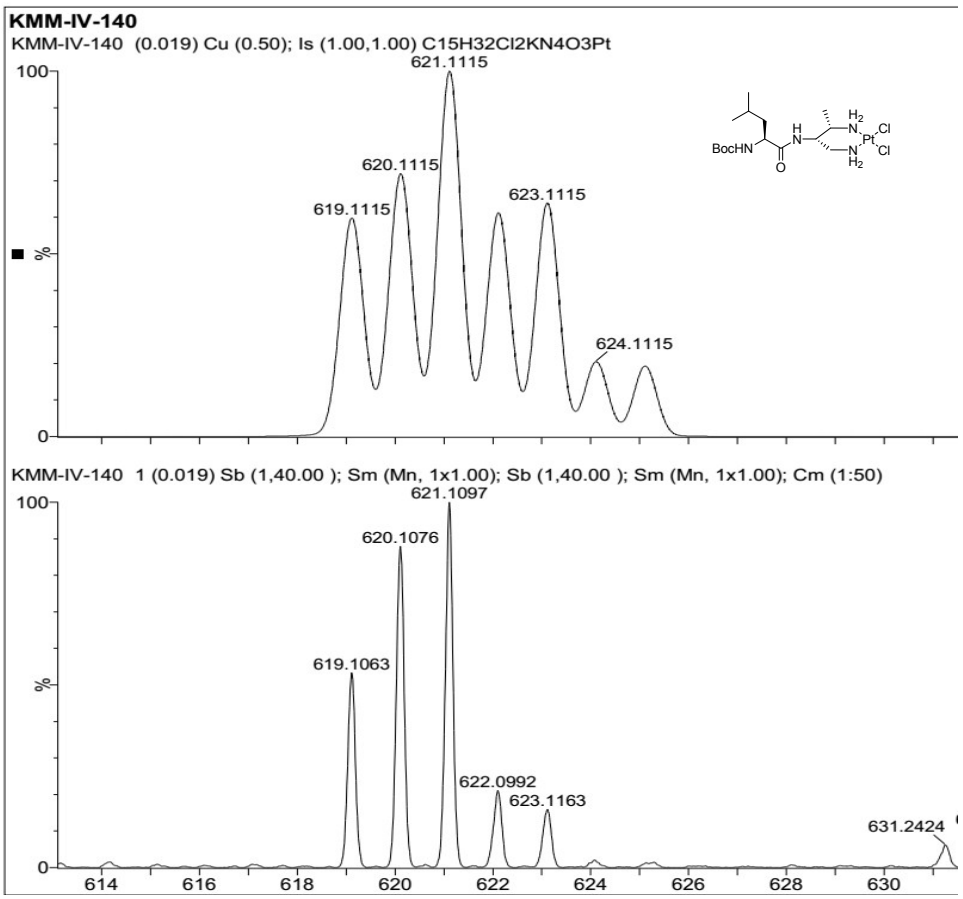
S28: ^{13}C NMR of Diazide **6c** (precursor of **7c**; 125 MHz, $CDCl_3$).



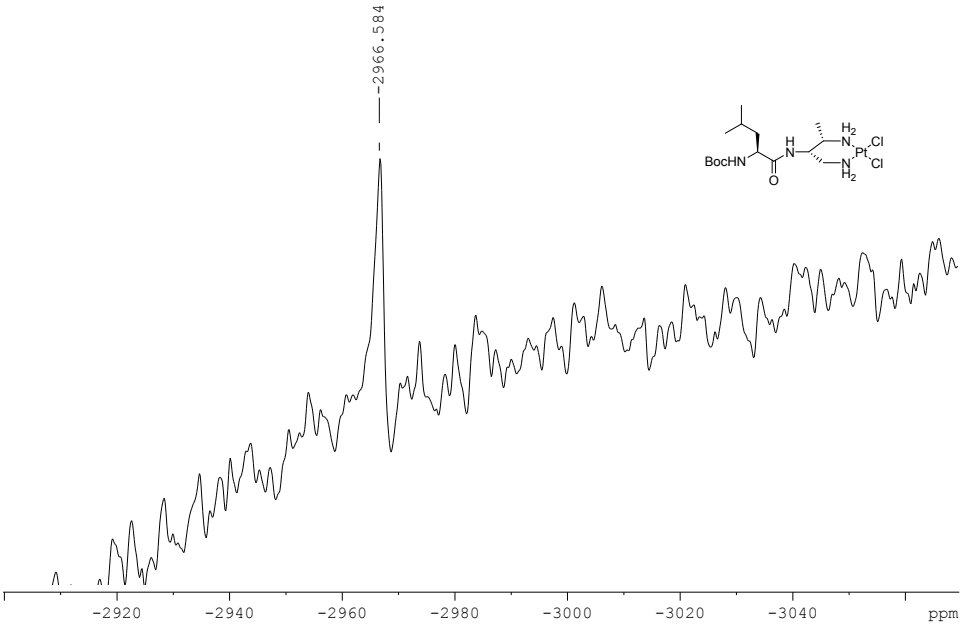
S29: HRMS of compound **7c**.



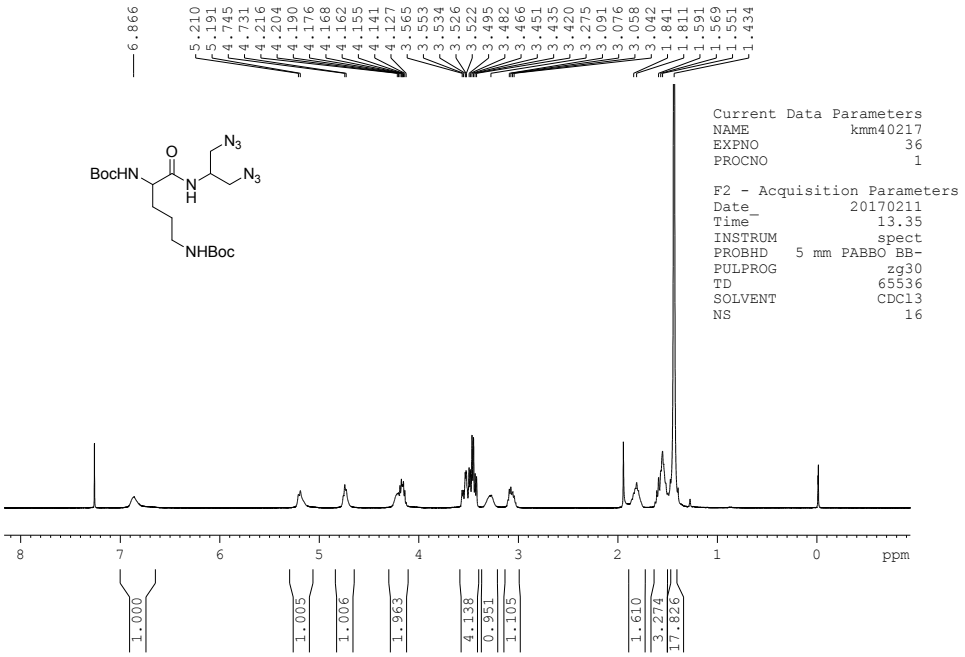
S30: ESI-MS spectrum of **7c**, peaks corresponding to $[M\text{-}Cl+CH_3CN]^+$, m/z 587, and $[M+K]^+$, m/z 620.



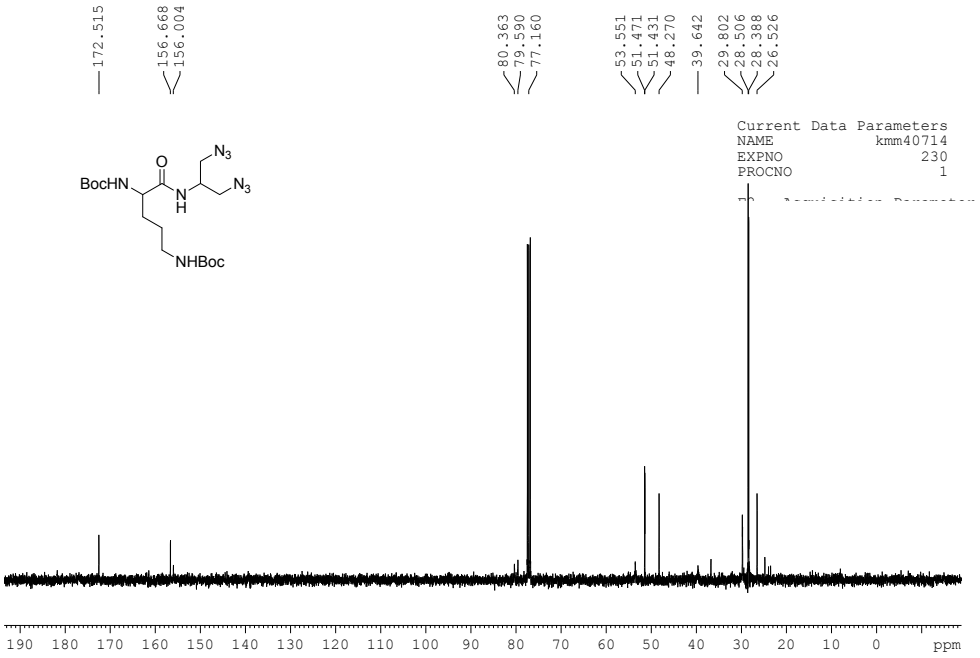
S31: isotopic pattern of **7c** corresponding to $[M+K]^+$, theoretical (above), experimental (below)



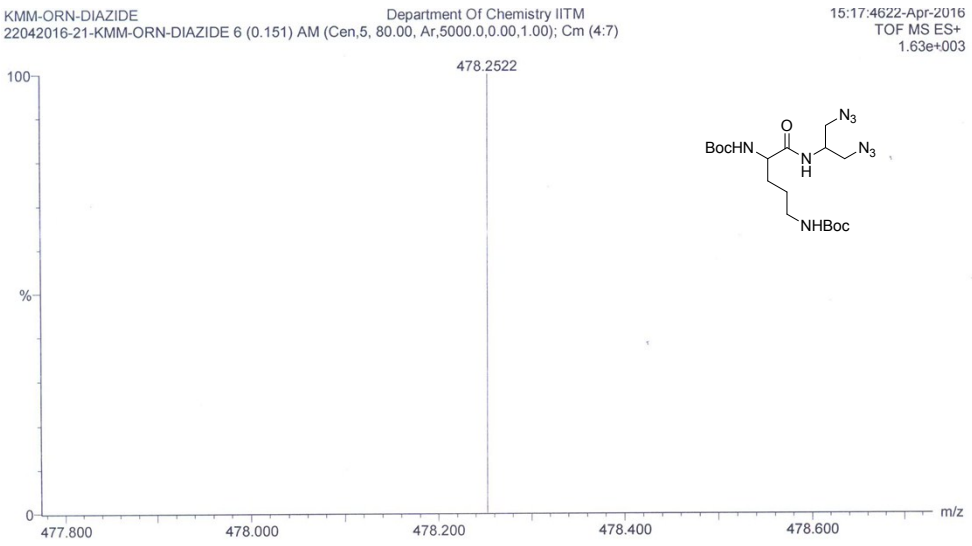
S32: ^{195}Pt NMR of compound **7c**. (107 MHz, DMSO-d_6)



S33: ^{1}H NMR of diazide **6d** (precursor of **7d**; 400 MHz, $CDCl_3$)

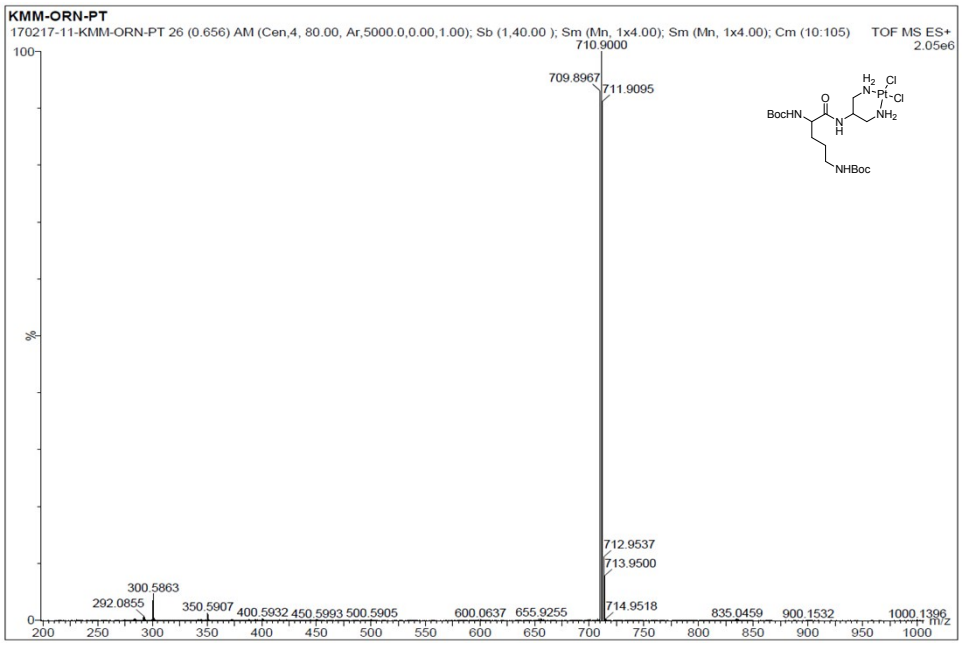


S34: ^{13}C NMR of diazide **6d** (precursor of **7d**; 100 MHz, $CDCl_3$)

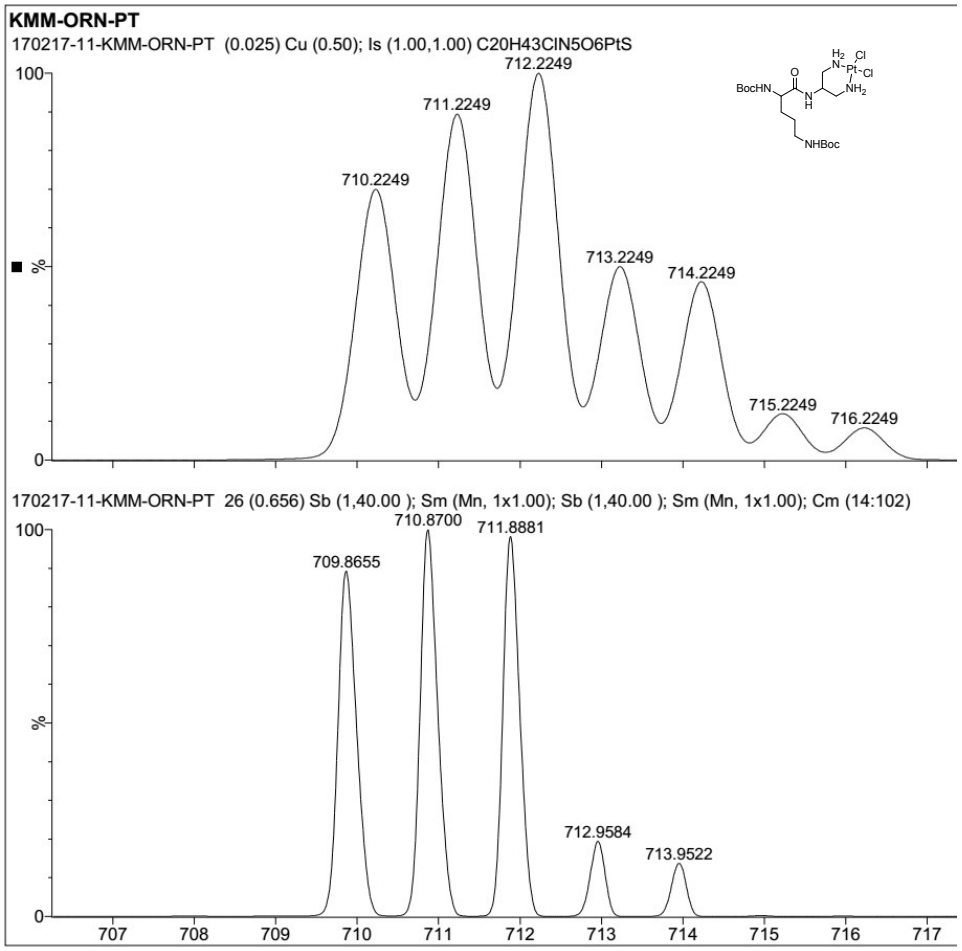


Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
478.2522	478.2502	2.0	4.2	6.5	n/a	C18 H33 N9 O5 Na

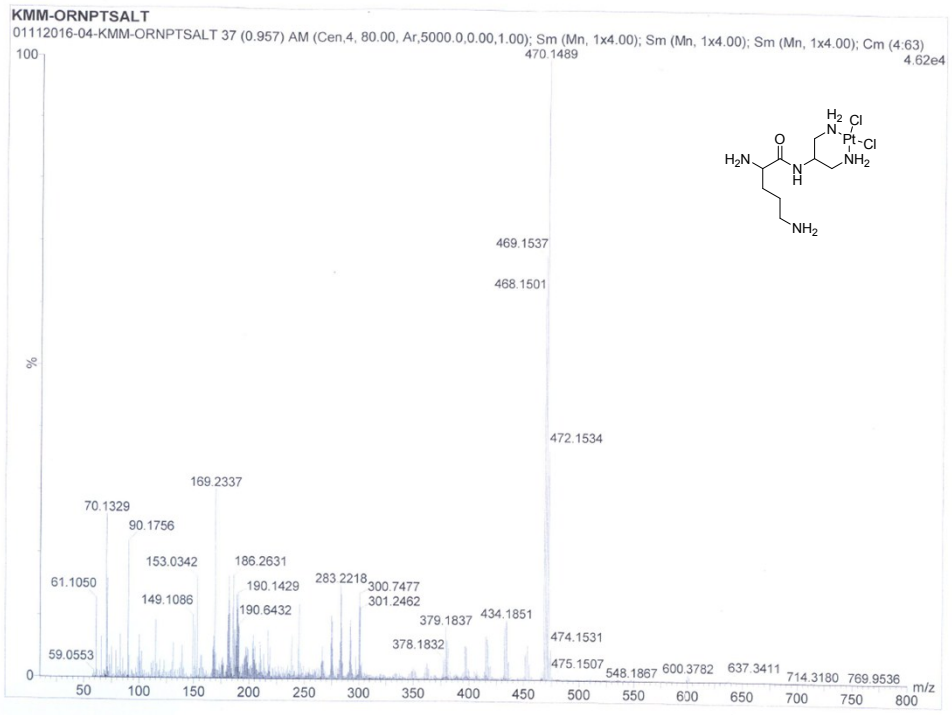
S35: HRMS of diazide **6d** (precursor of **7d**).



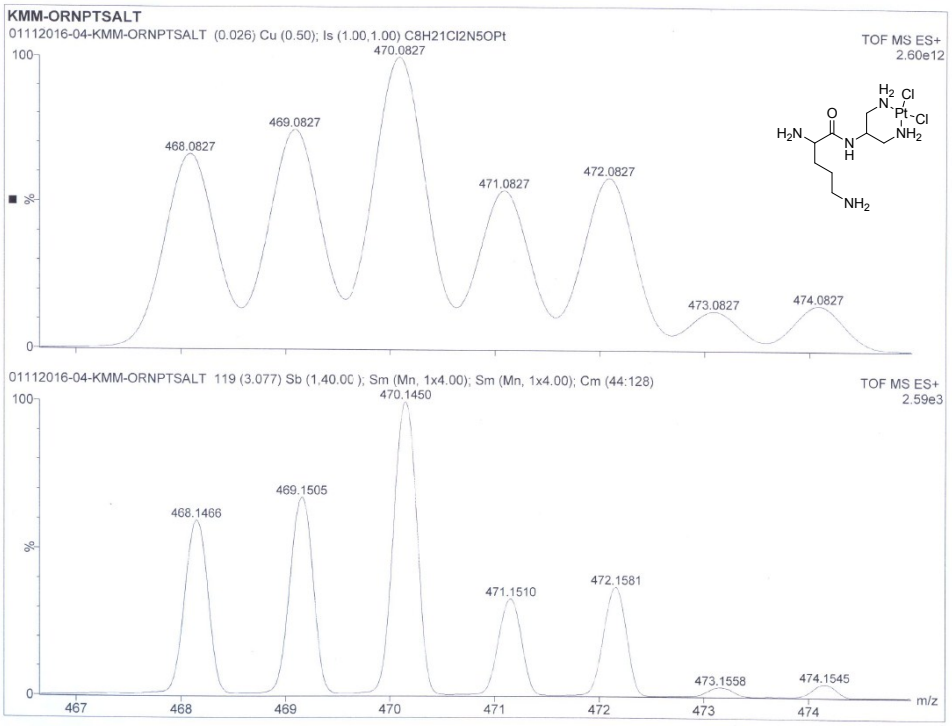
S36: ESI-MS spectrum of **7d**.



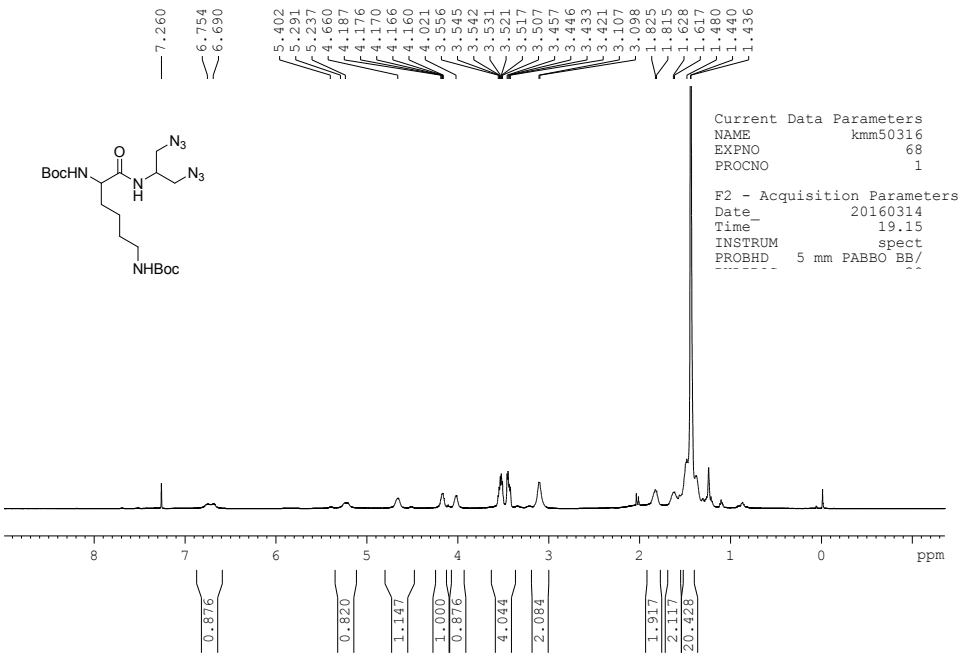
S37 Isotopic pattern of **7d** corresponding to $[M\text{-}Cl+DMSO]^+$ theoretical (above) and experimental (below).



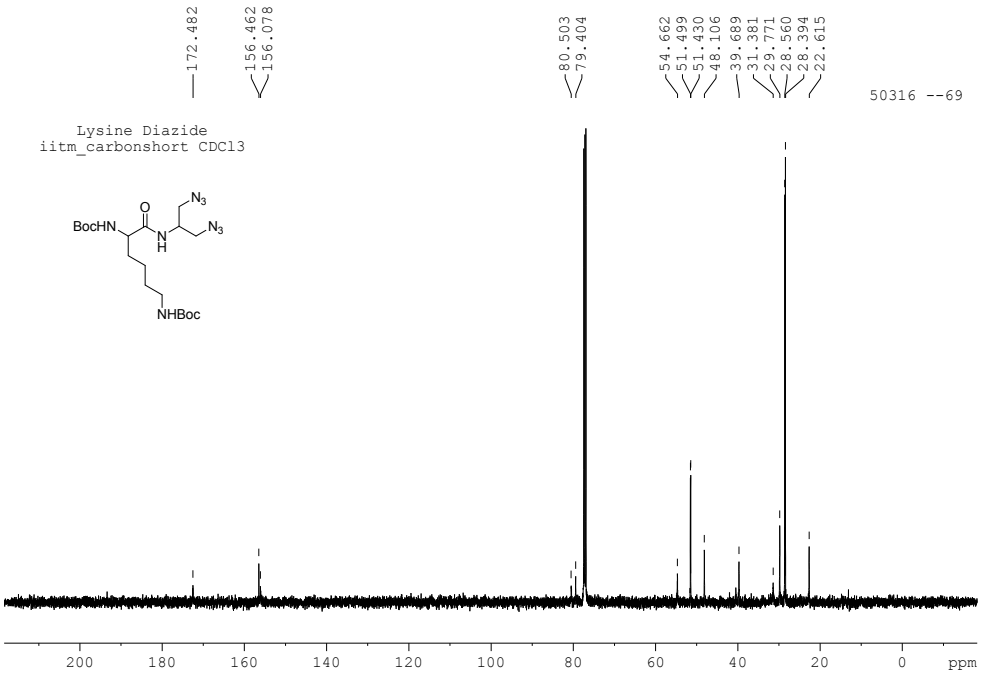
S38: ESI-MS spectrum of **7f**.



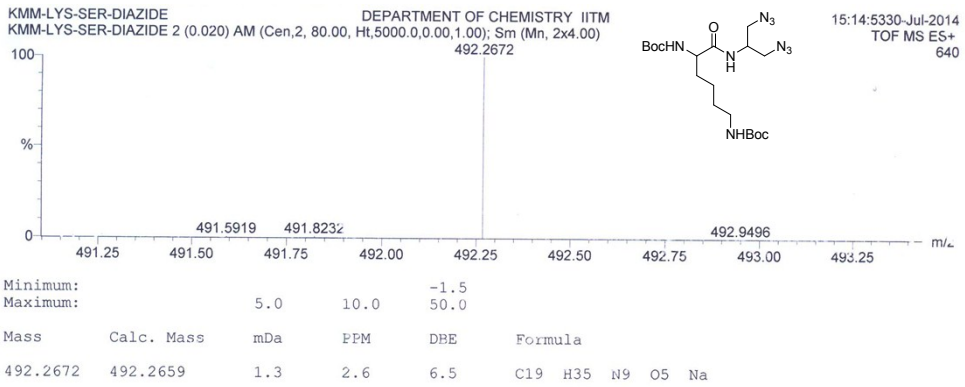
S39: isotopic pattern of **7f** corresponding to **[**M+H]$^+$ theoretical (above) and experimental (below)



S40: ^{1}H NMR of **6e** (500 MHz, $CDCl_3$)

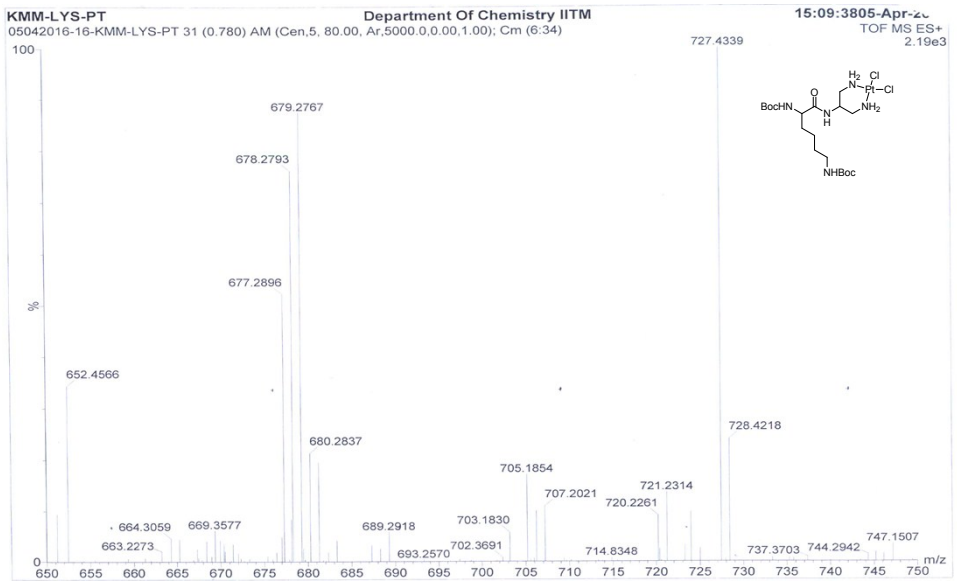


S41: ^{13}C NMR of **6e** (125 MHz, $CDCl_3$)

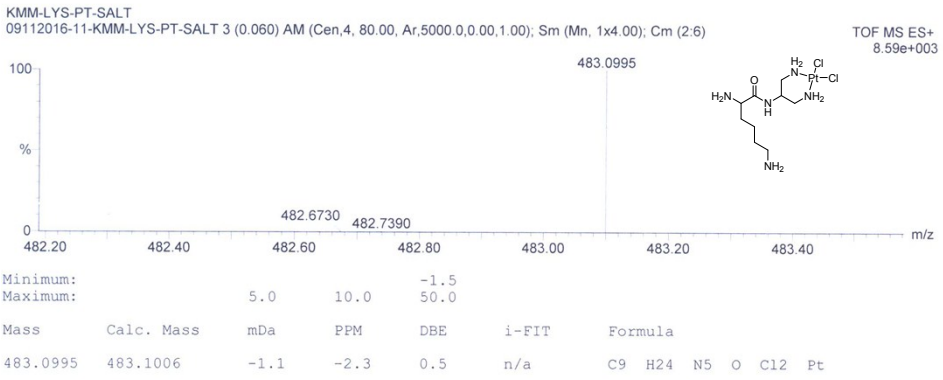


Minimum:				-1.5	
Maximum:		5.0	10.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
492.2672	492.2659	1.3	2.6	6.5	C19 H35 N9 O5 Na

S42: HRMS of **6e**.

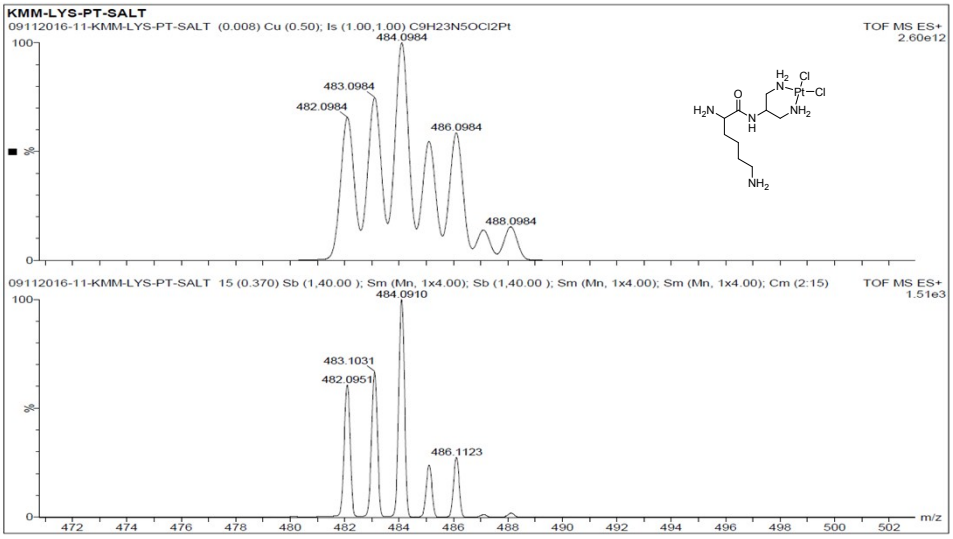


S43: ESI-MS spectrum of **7e**, m/z 678 $[M\text{-}Cl+CH_3OH]^+$ and corresponding isotopic pattern.

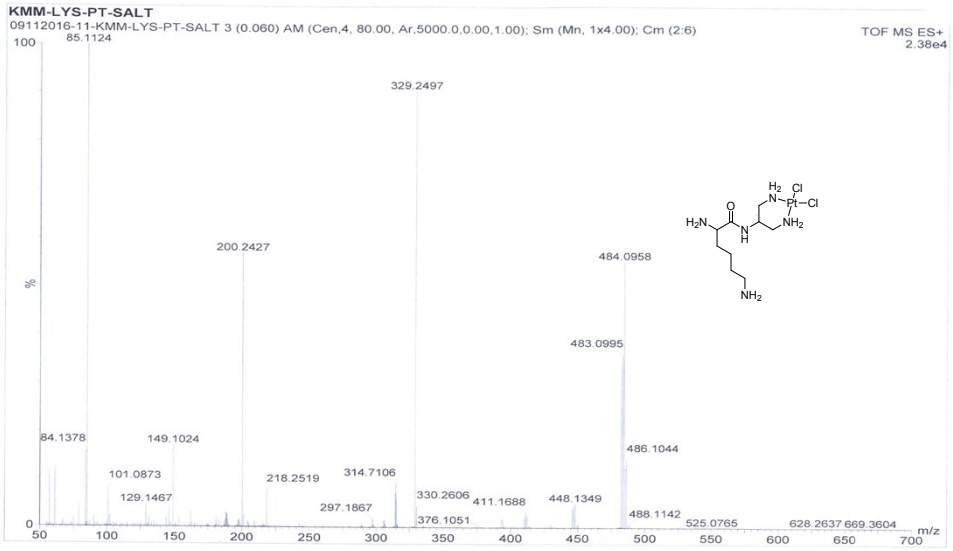


Minimum:			-1.5			
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
483.0995	483.1006	-1.1	-2.3	0.5	n/a	C9 H24 N5 O Cl2 Pt

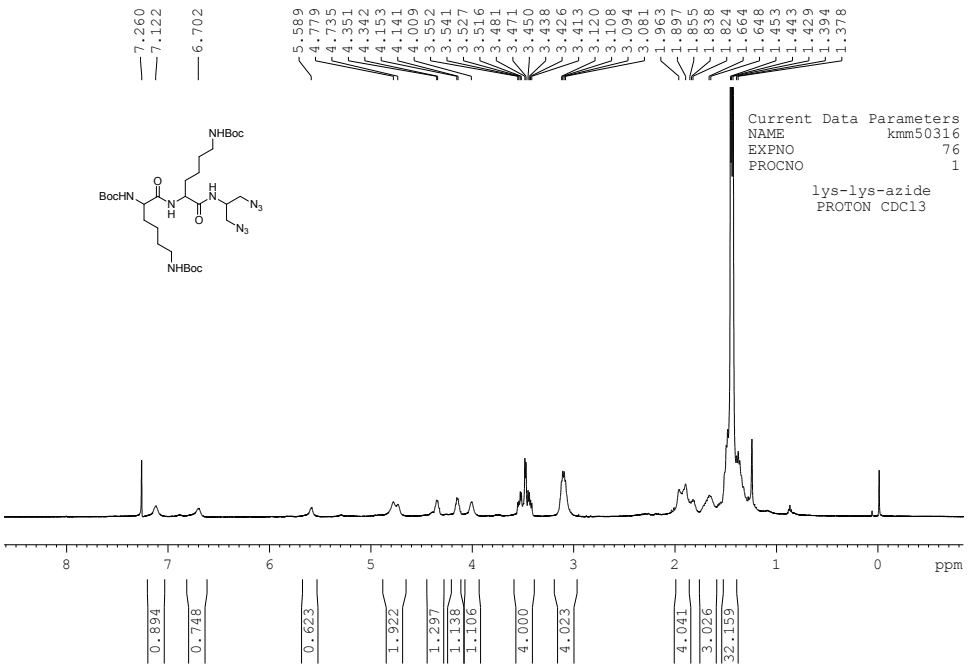
S44: HRMS of **7g**.



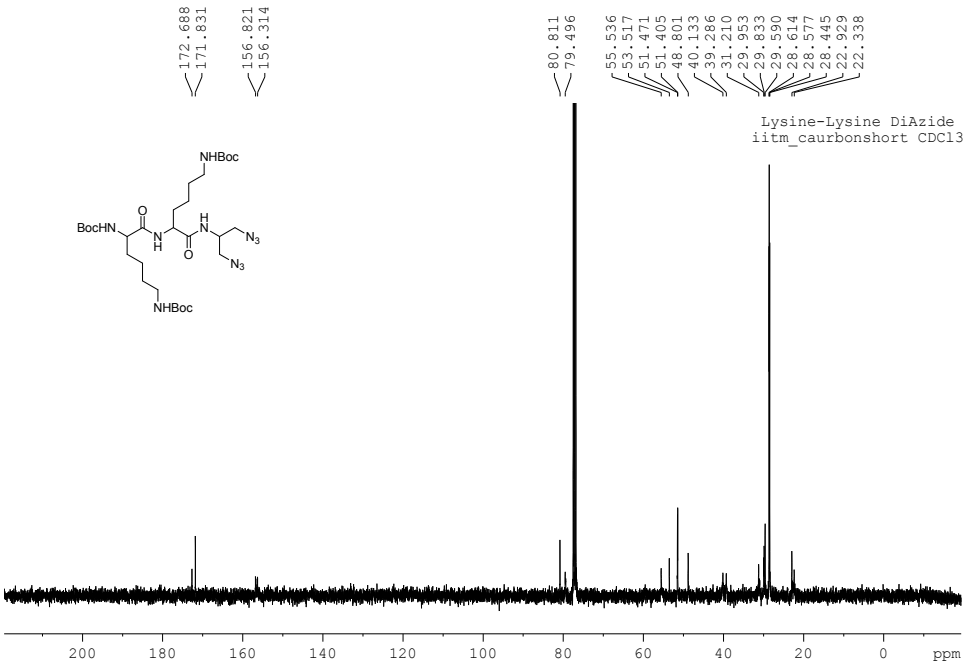
S45: isotopic pattern of **7g** $[M+H]^+$.



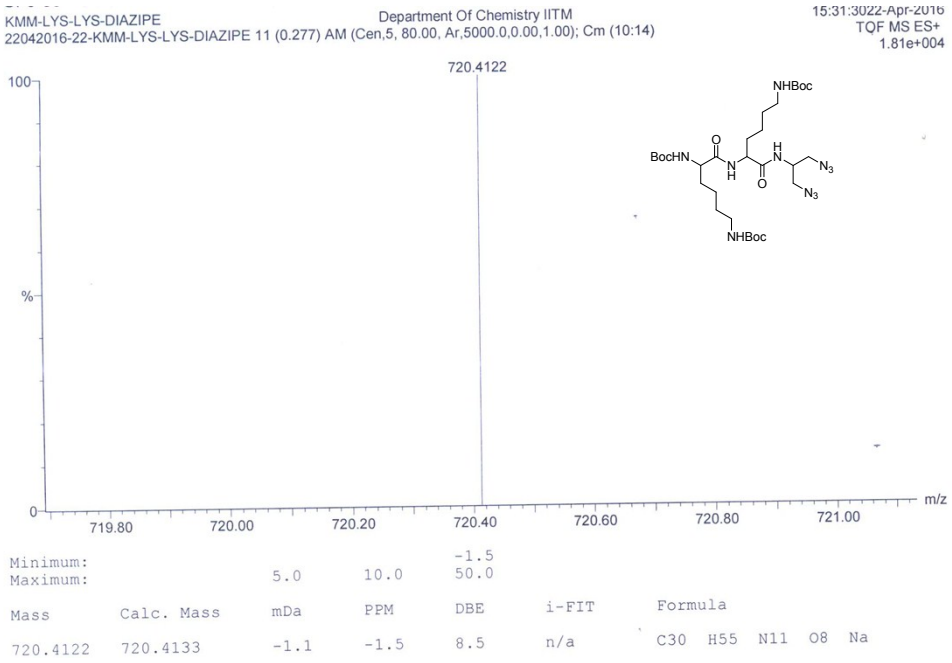
S46: ESI-MS spectrum of **7g**.



S47: ^{1}H NMR of diazide **6h** (precursor of **7h**; 500 MHz, $CDCl_3$)

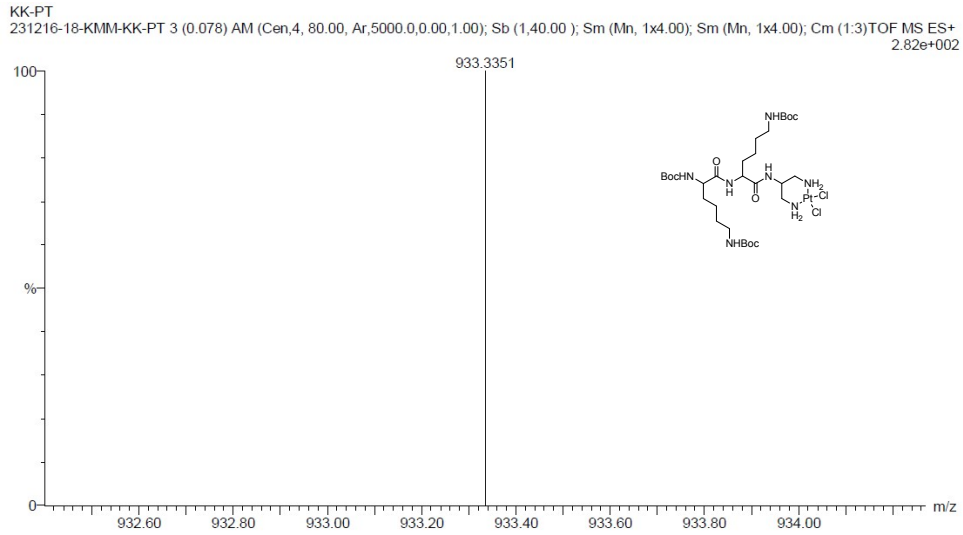


S48: ^{13}C NMR of diazide **6h** (precursor of **7h**; 125 MHz, $CDCl_3$)

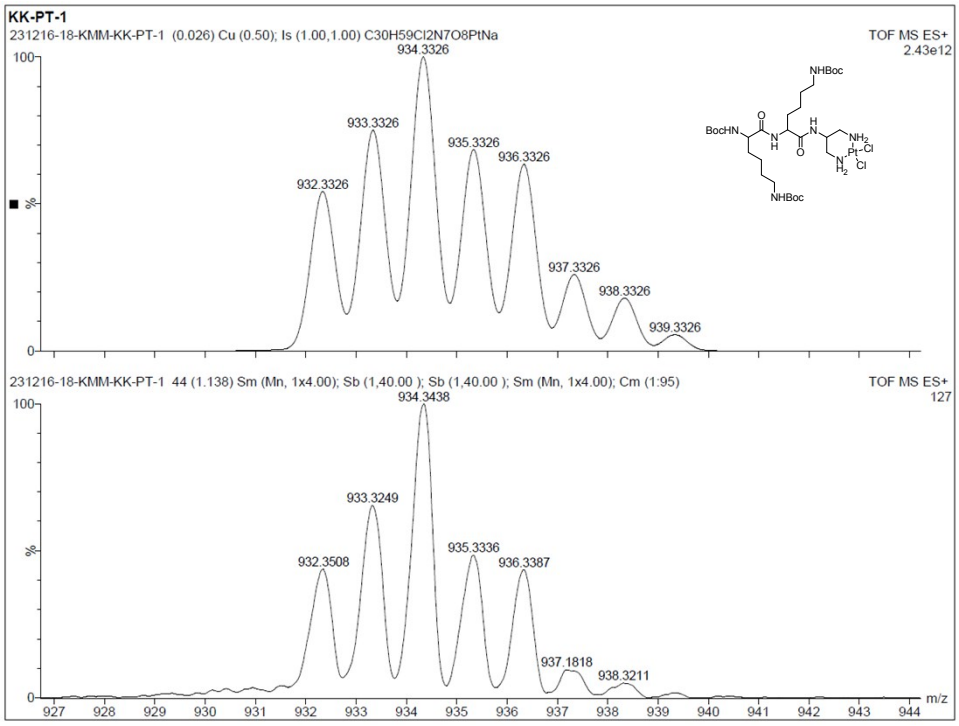


Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
720.4122	720.4133	-1.1	-1.5	8.5	n/a	C30 H55 N11 O8 Na

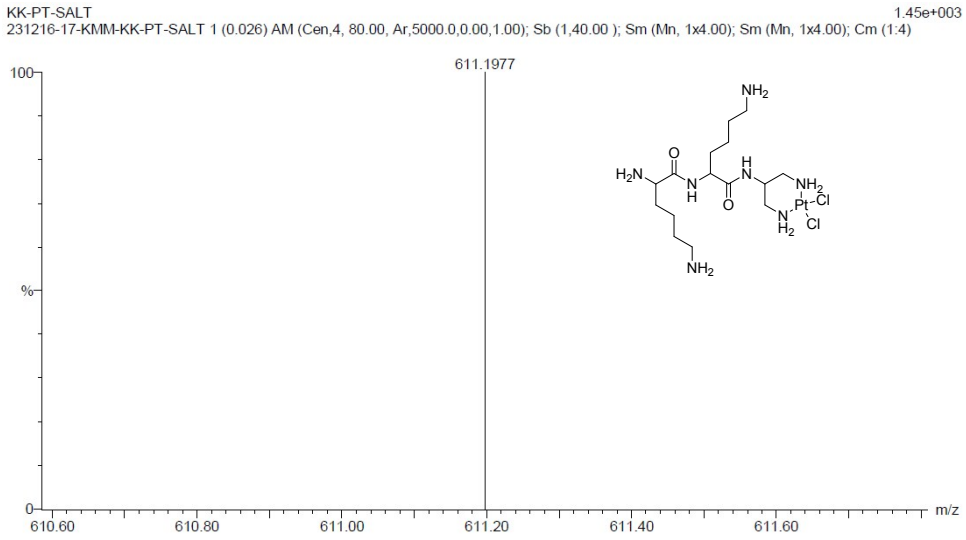
S49: HRMS of **6h**.



S50: HRMS of **7h**.

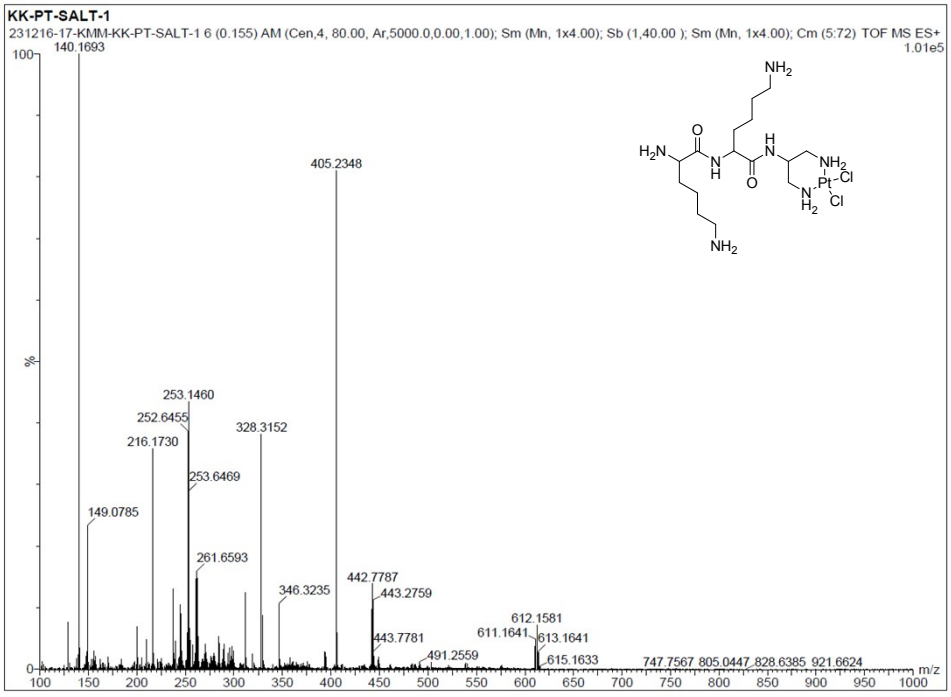


S51: Isotopic pattern of **7h** corresponding to $[M+Na]^+$ theoretical (above) experimental (below).

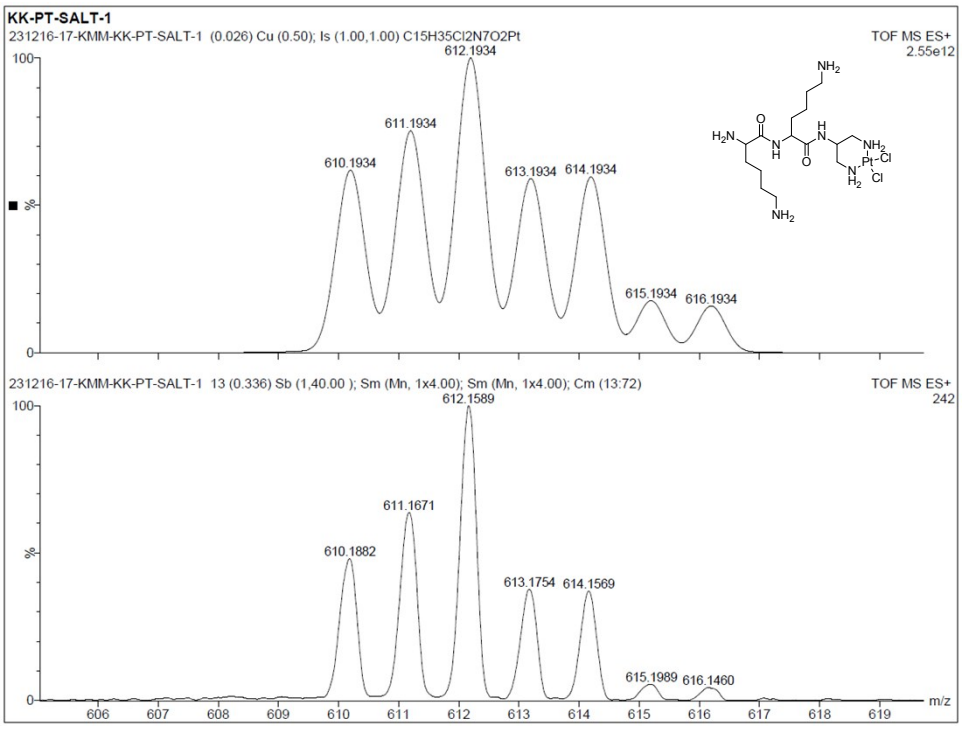


Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
611.1977	611.1955	2.2	3.6	1.5	n/a	C15 H36 N7 O2 Cl2 Pt

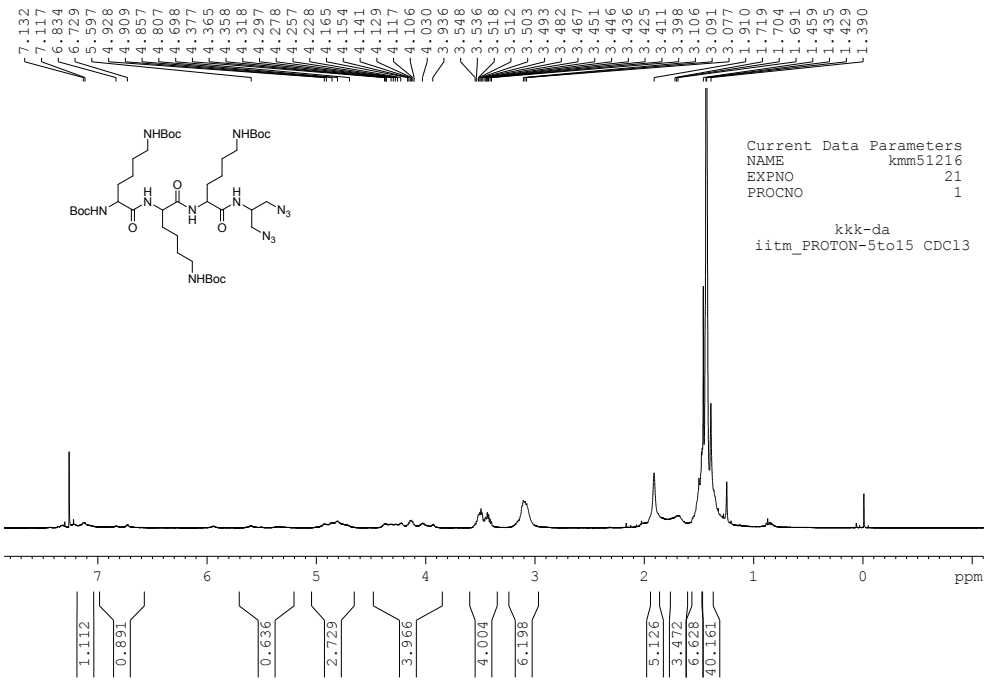
S52: HRMS of **7i**.



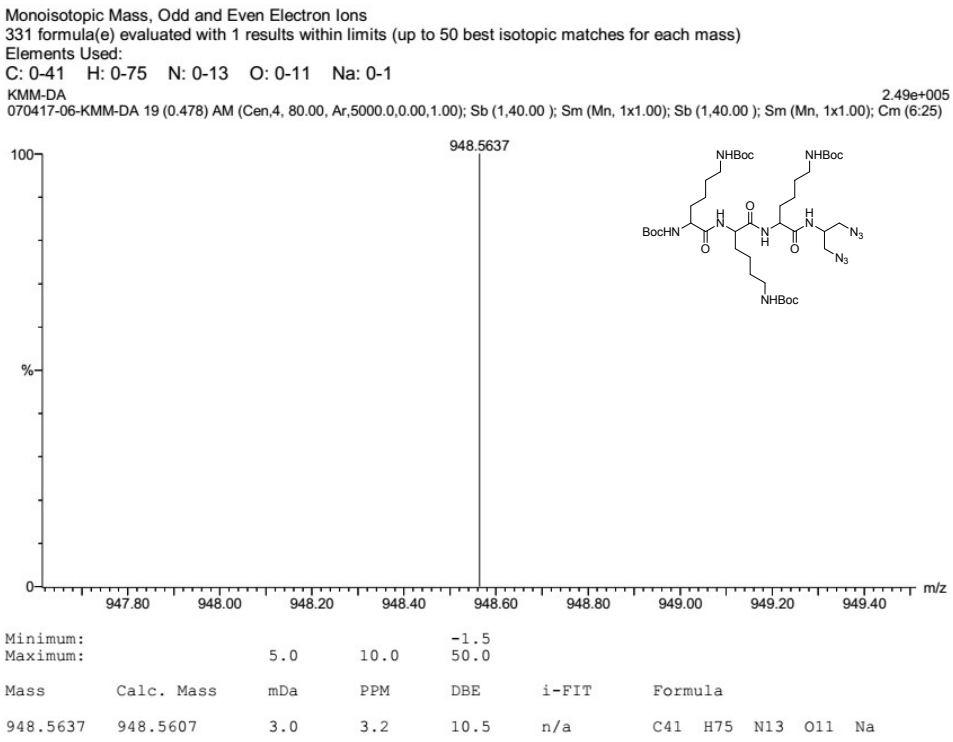
S53: ESI-MS spectrum of **7i**.



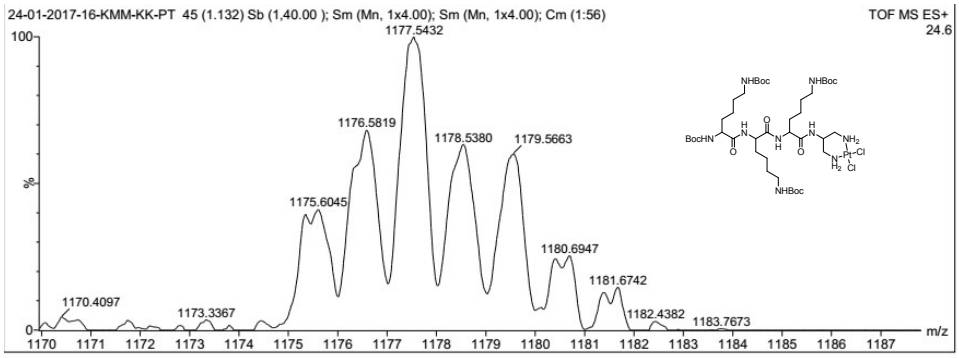
S54: MS isotopic pattern of **7i** corresponding to $[M+H]^+$, theoretical (above) and experimental (below).



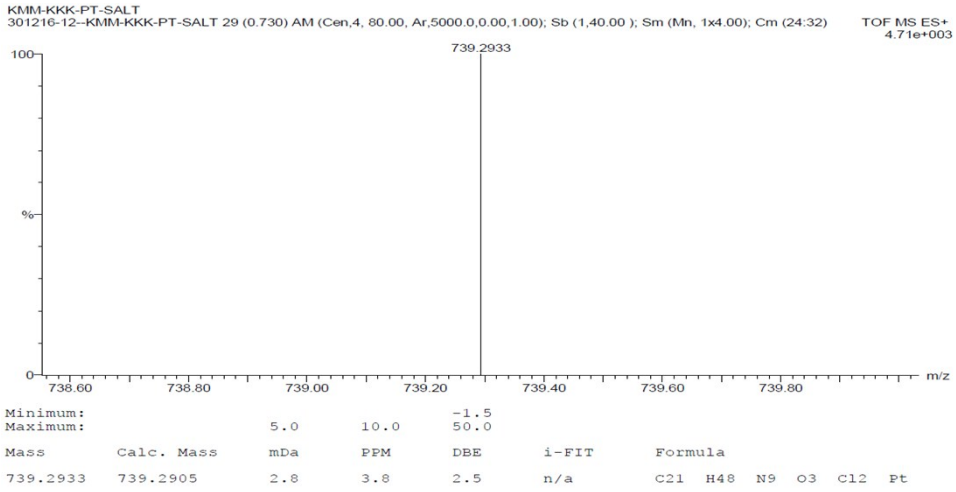
S55: ^{1}H NMR of diazide **6j** (precursor of **7j**; 500 MHz, $CDCl_3$)



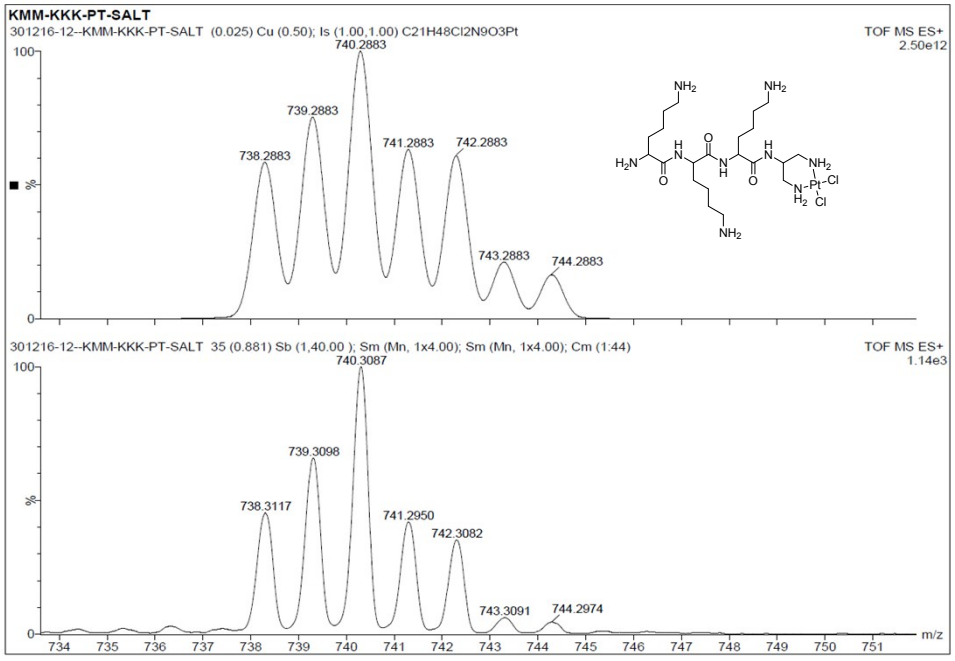
S56: HRMS of **6j** (precursor of **7j**).



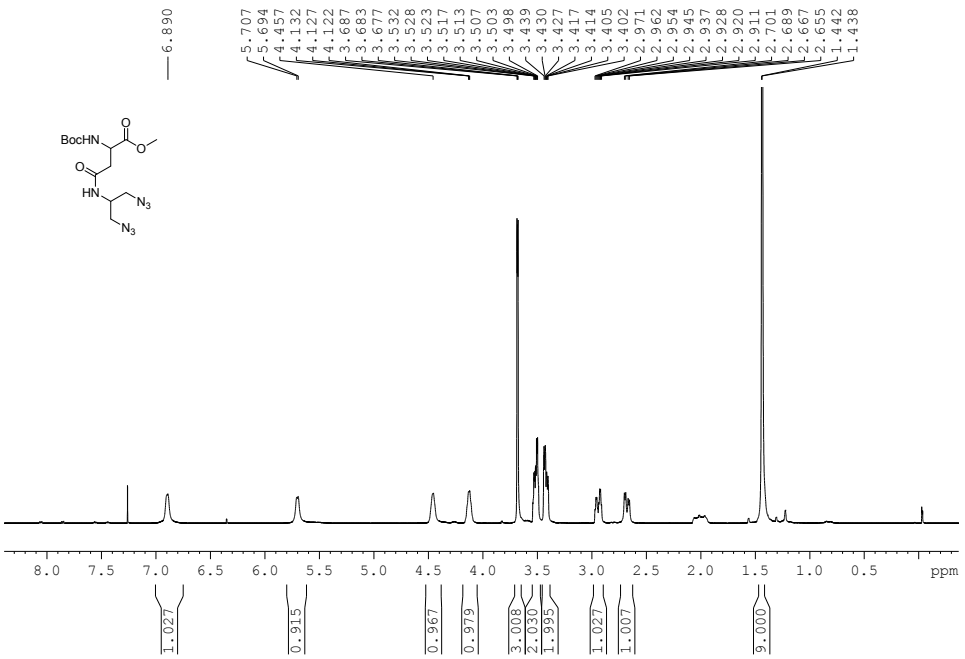
S57: ESI-MS isotopic pattern of **7j** m/z 1177, $[M+K]^+$



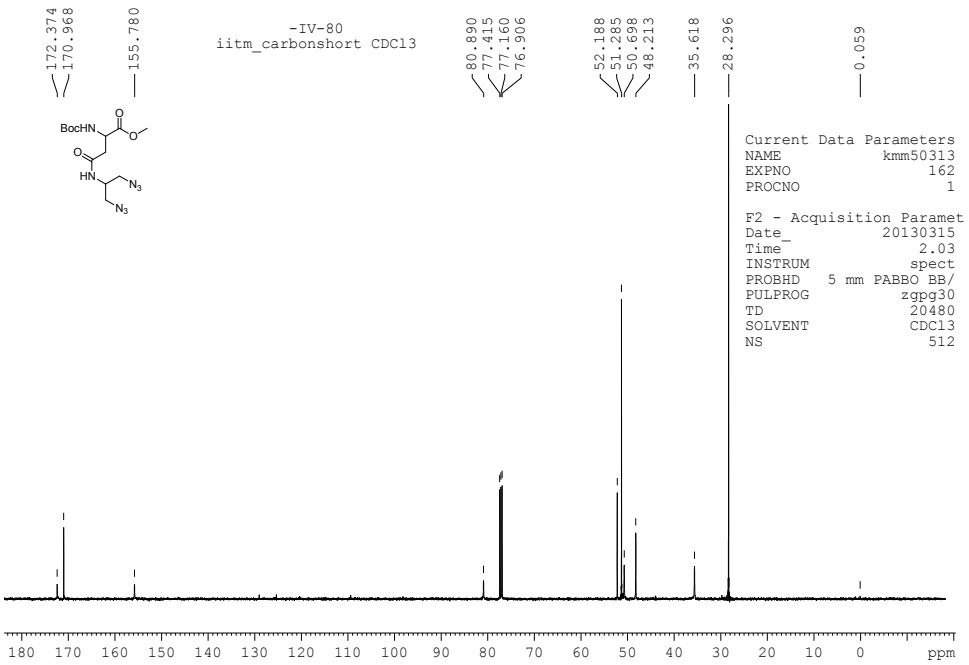
S58: HRMS of **7k**.



S59: isotopic pattern of **7k** corresponding to $[M+H]^+$, theoretical (above) and experimental (below).



S60: ^{1}H NMR of **12** (500 MHz, $CDCl_3$).



S61: ^{13}C NMR of **12** (125 MHz, $CDCl_3$).

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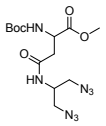
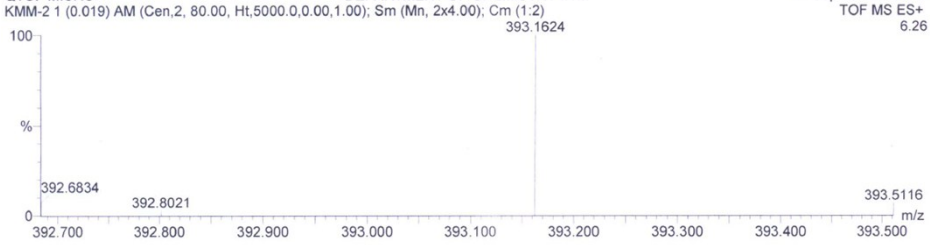


Single Mass Analysis
Tolerance = 200.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
105 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

QTOF MICRO DEPARTMENT OF CHEMISTRY IITM 17-Sep-201211:56:19
KMM-2 1 (0.019) AM (Cen,2, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x4.00); Cm (1:2) TOF MS ES+
6.26



Minimum:				-1.5		
Maximum:		200.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
393.1624	393.1611	1.3	3.3	6.5	1	C13 H22 N8 O5 Na

S62: HRMS of **12**.

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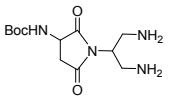
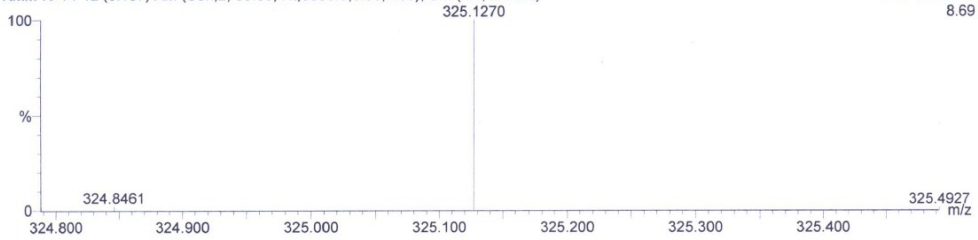


Single Mass Analysis
Tolerance = 200.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

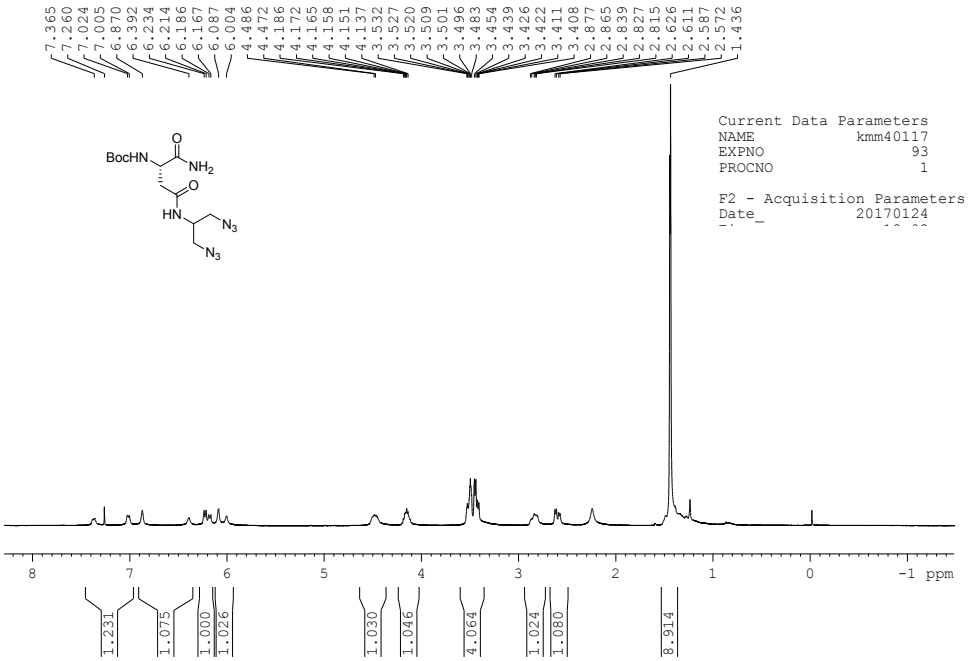
Monoisotopic Mass, Odd and Even Electron Ions
48 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

QTOF MICRO DEPARTMENT OF CHEMISTRY IITM 20-Feb-201308:41:39
KMM-IV-71 12 (0.197) AM (Cen,2, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x4.00) TOF MS ES+
8.69

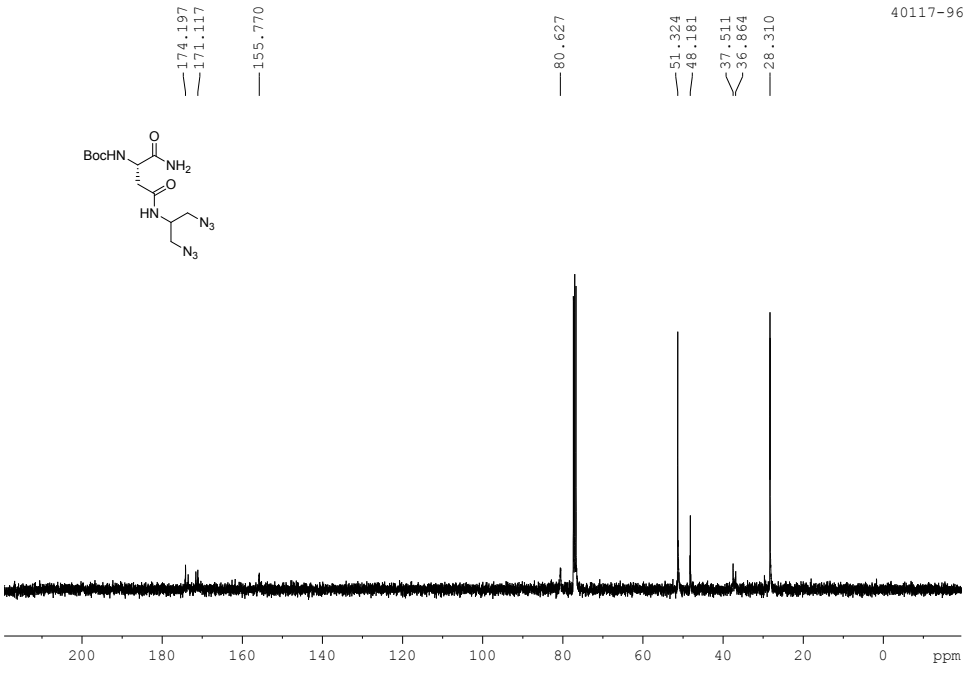


Minimum:				-1.5		
Maximum:		200.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
325.1270	325.1278	-0.9	-2.6	3.5	1	C12 H22 N4 O4 K

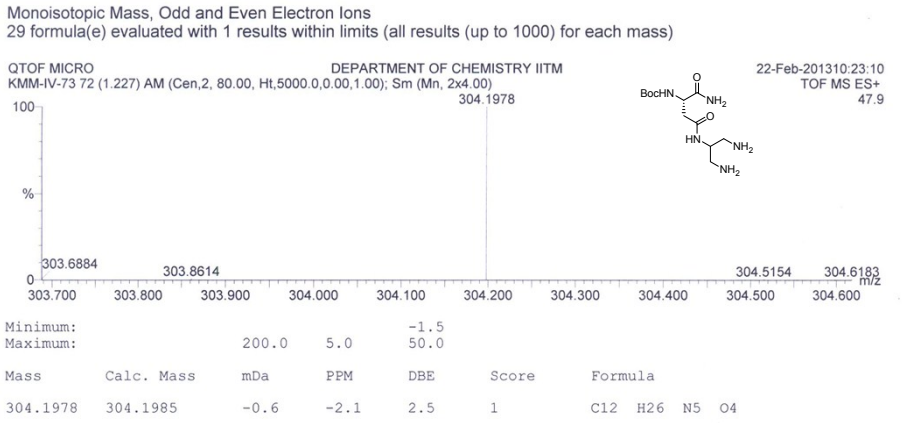
S63: HRMS of **13**.



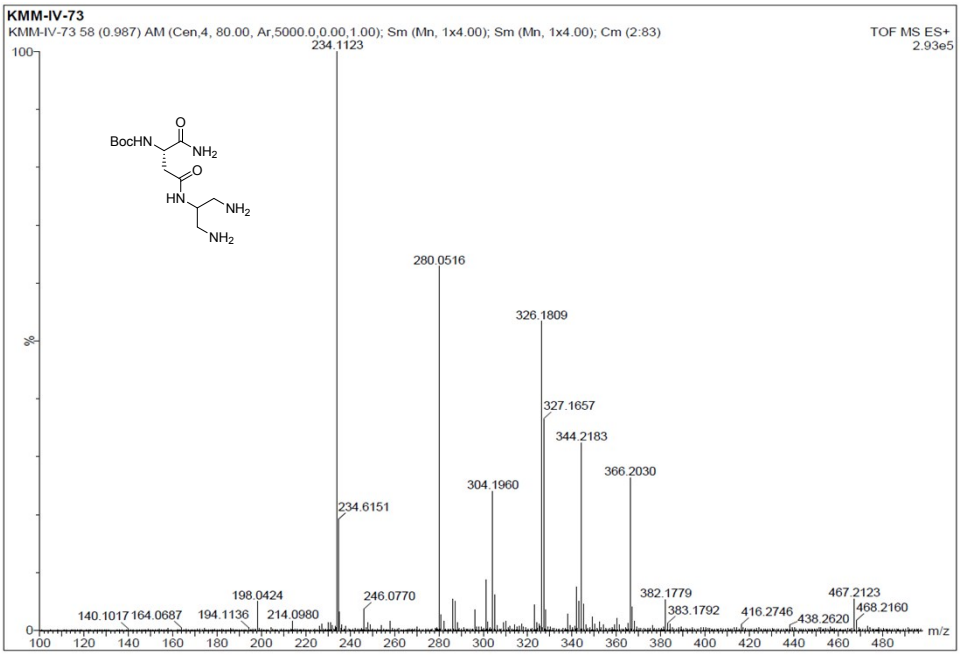
S64: ^{1}H NMR of **18**. (400 MHz, $CDCl_3$).



S65: ^{13}C NMR of **18**. (100MHz, $CDCl_3$).



S66: HRMS of diamine prepared through reduction of **18**.



S67: ESI-MS spectrum of diamine prepared through reduction of **18**.

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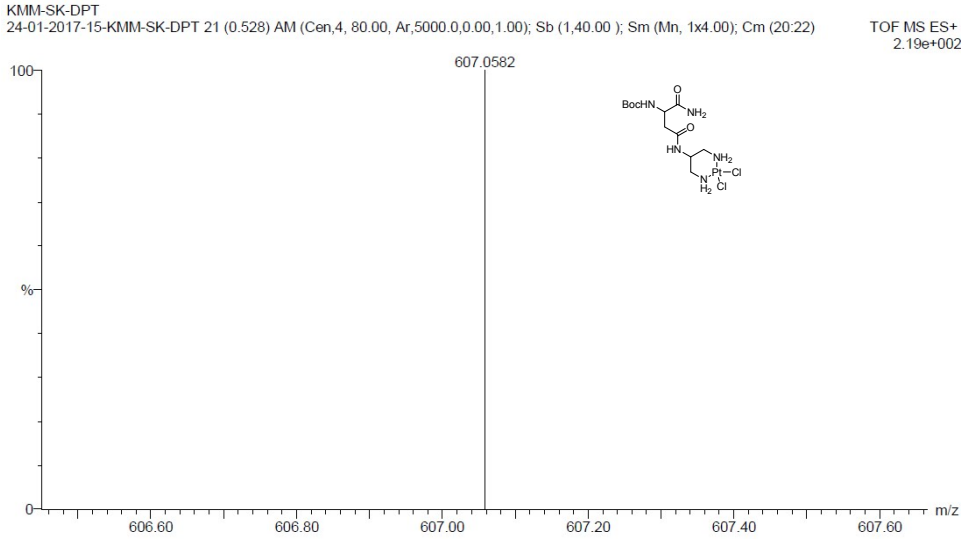
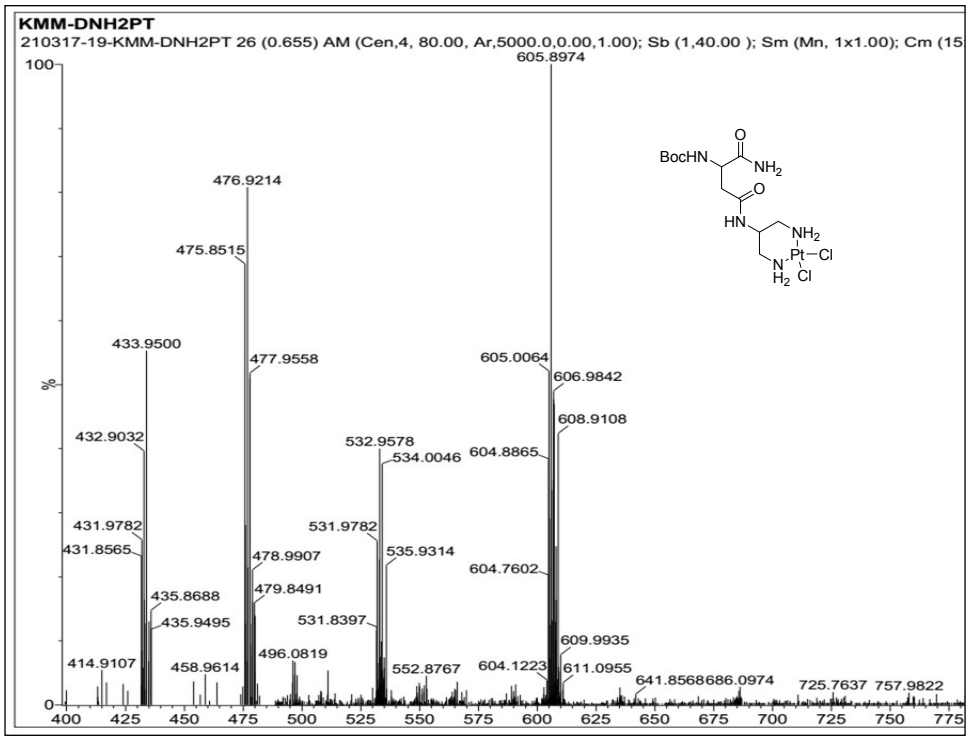
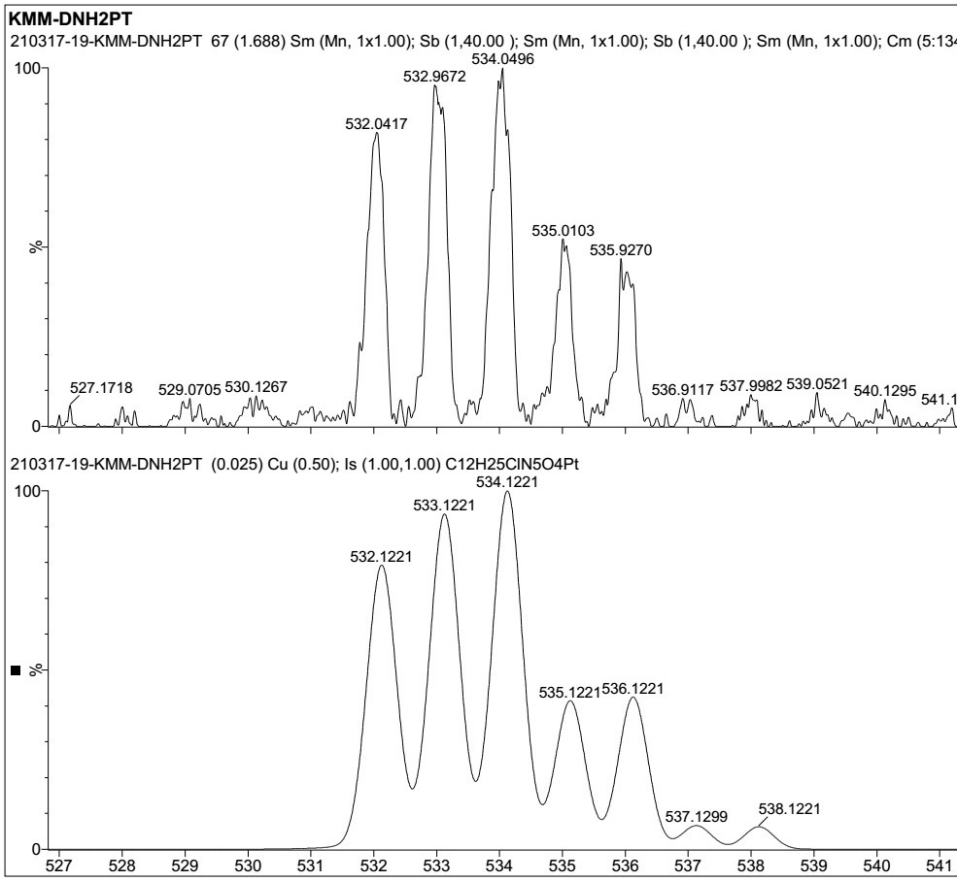


Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
607.0582	607.0569	1.3	2.1	2.5	n/a	C12 H25 N5 O4 Cl2 Pt K

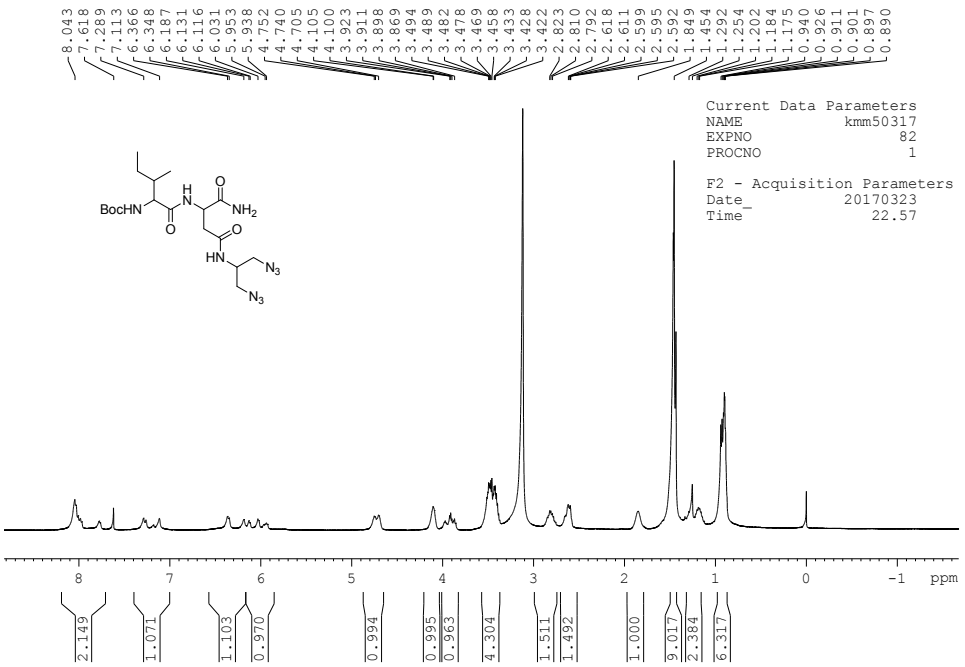
S68: HR-MS of **19**.



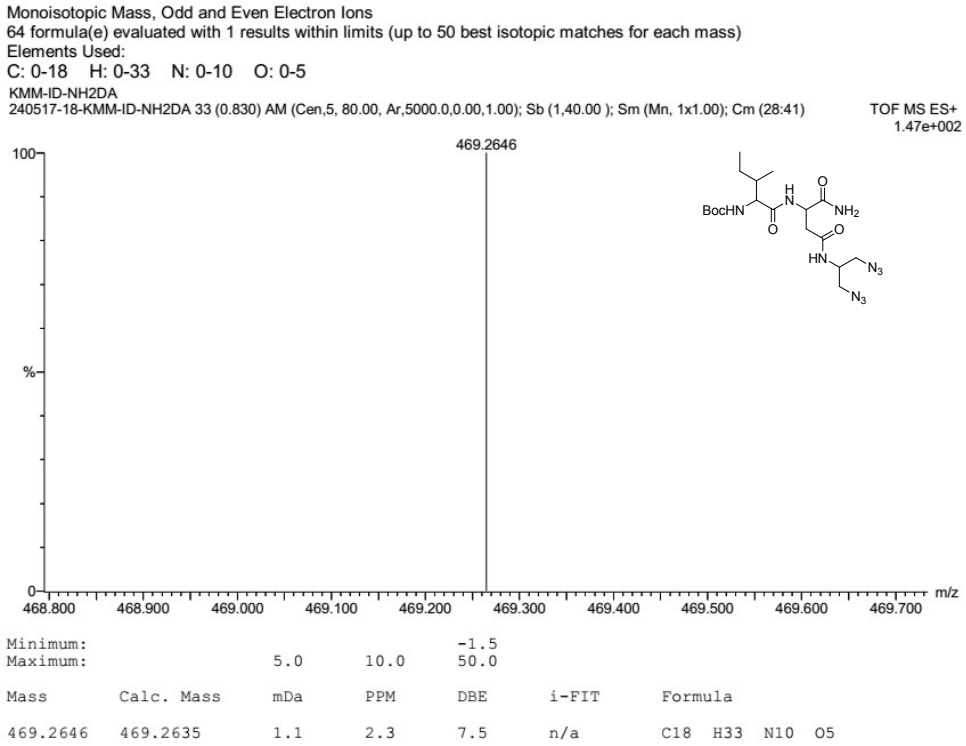
S69: ESI-MS of **19**.



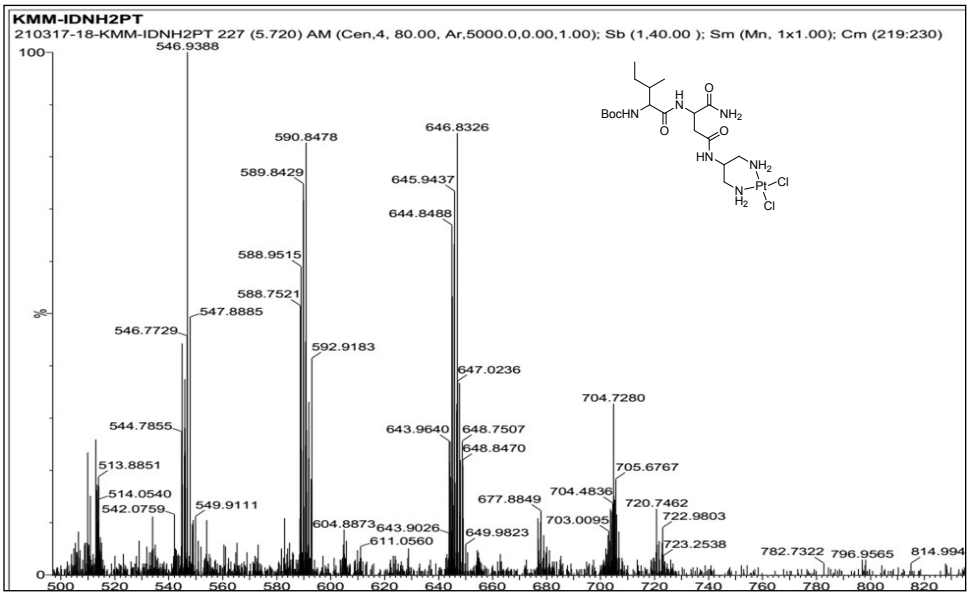
Isotopic pattern of **19** corresponding to $[M\text{-}Cl]^+$ theoretical (below) and experimental (above).



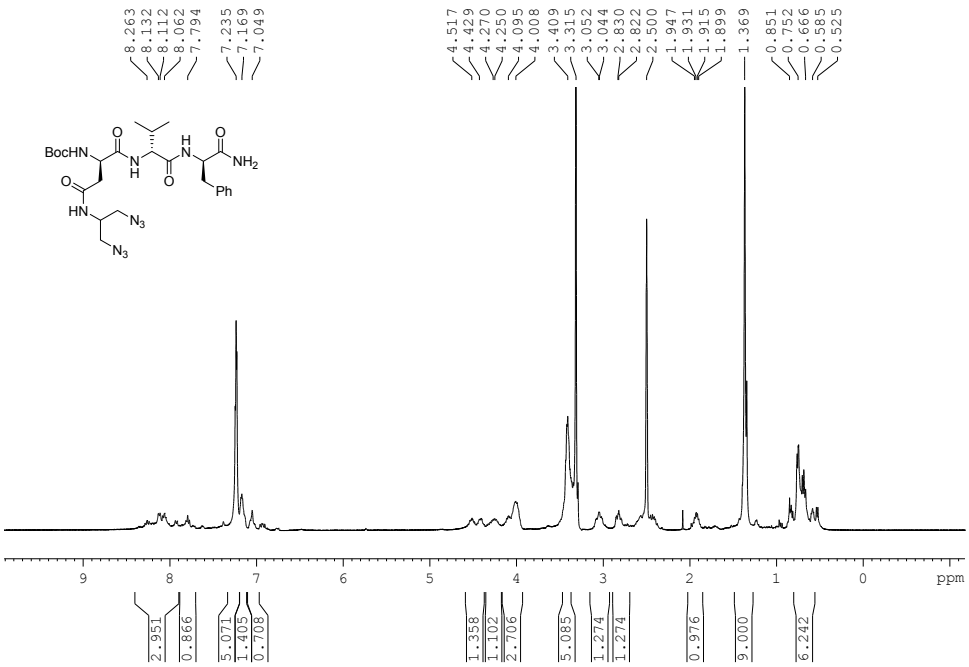
S70: ^{1}H NMR of diazide **20** (500 MHz, $CDCl_3$ (trace amount of DMSO-d_6))



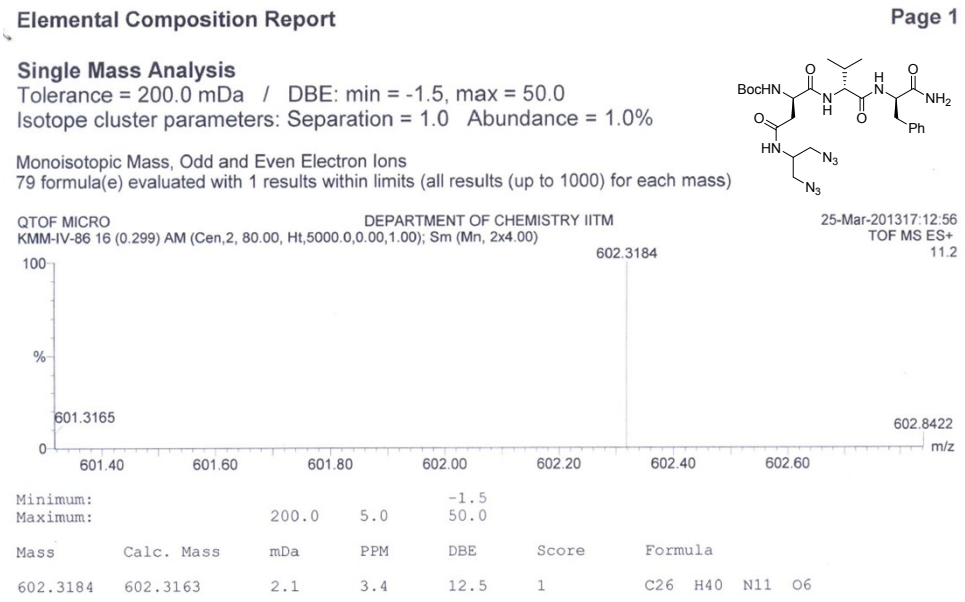
S71: HRMS of **20**.



S72: ESI-MS spectrum of **21**



S73: ^{1}H NMR of 25 (400 MHz, $CDCl_3$).



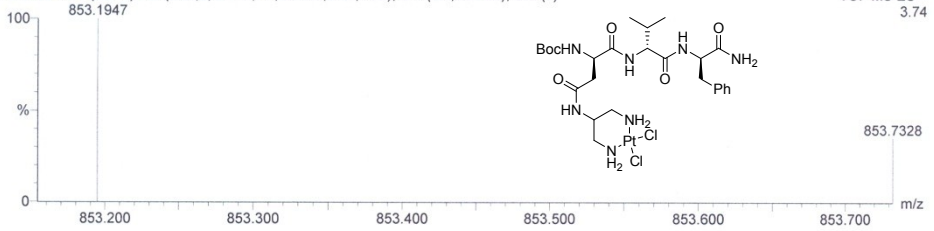
S74: HRMS of **25**.

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 200.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

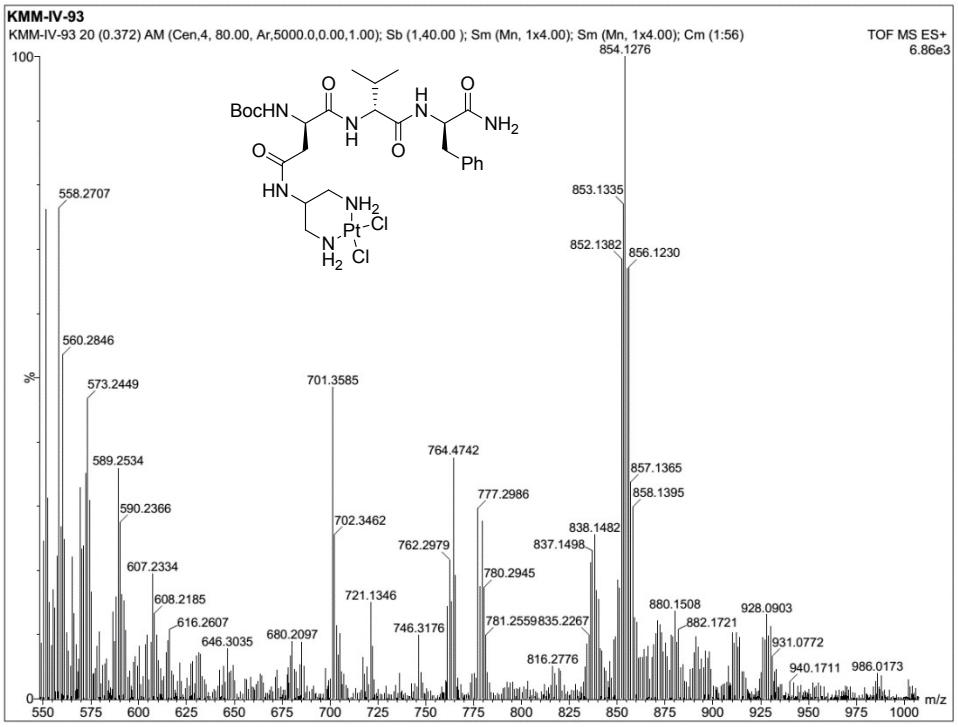
Monoisotopic Mass, Odd and Even Electron Ions
670 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

QTOF MICRO DEPARTMENT OF CHEMISTRY IITM 06-Apr-201313:37:46
KMM-IV-93 1 (0.019) AM (Cen,2, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x4.00); Cm (1) TOF MS ES+
3.74

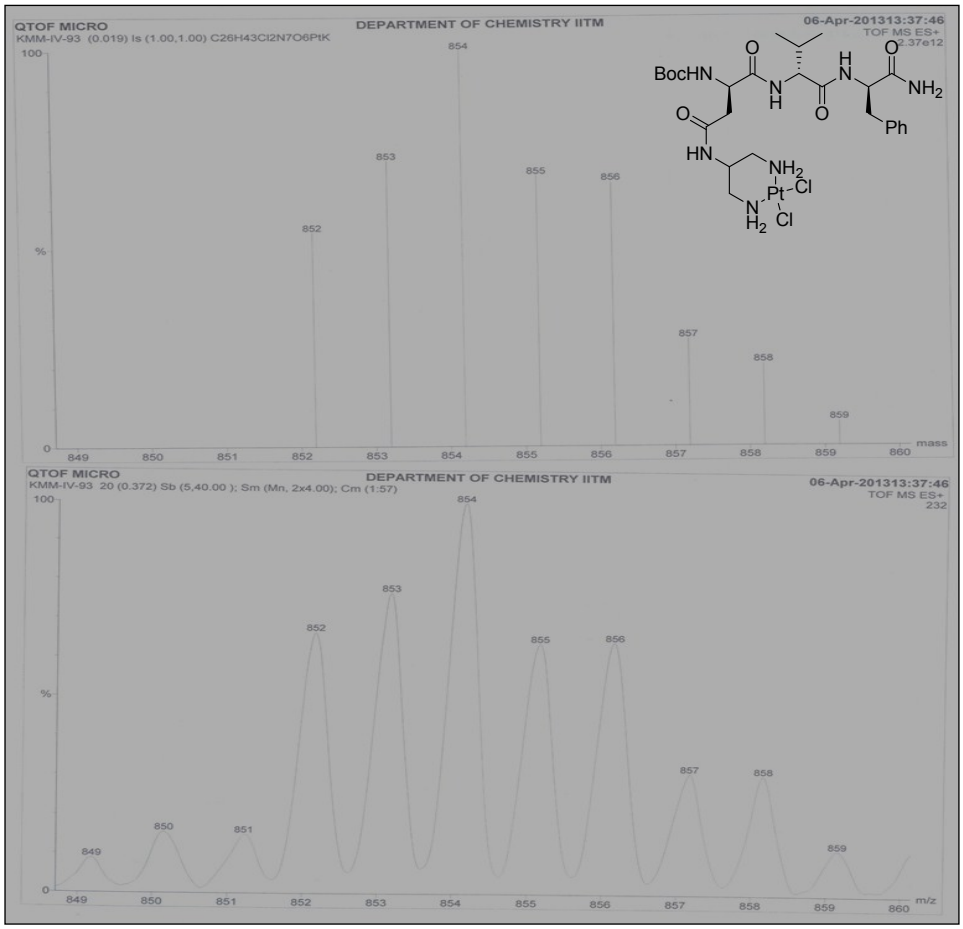


Minimum:				-1.5		
Maximum:		200.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
853.1947	853.1937	1.0	1.2	8.5	1	C26 H43 N7 O6 Cl2 Pt K

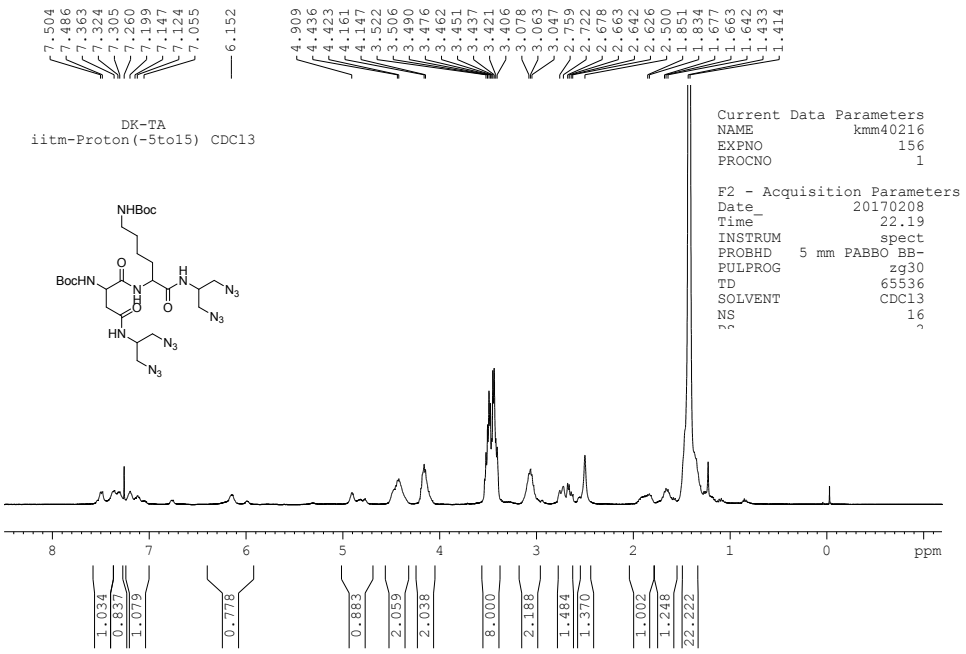
S75: HRMS of compound **26**.



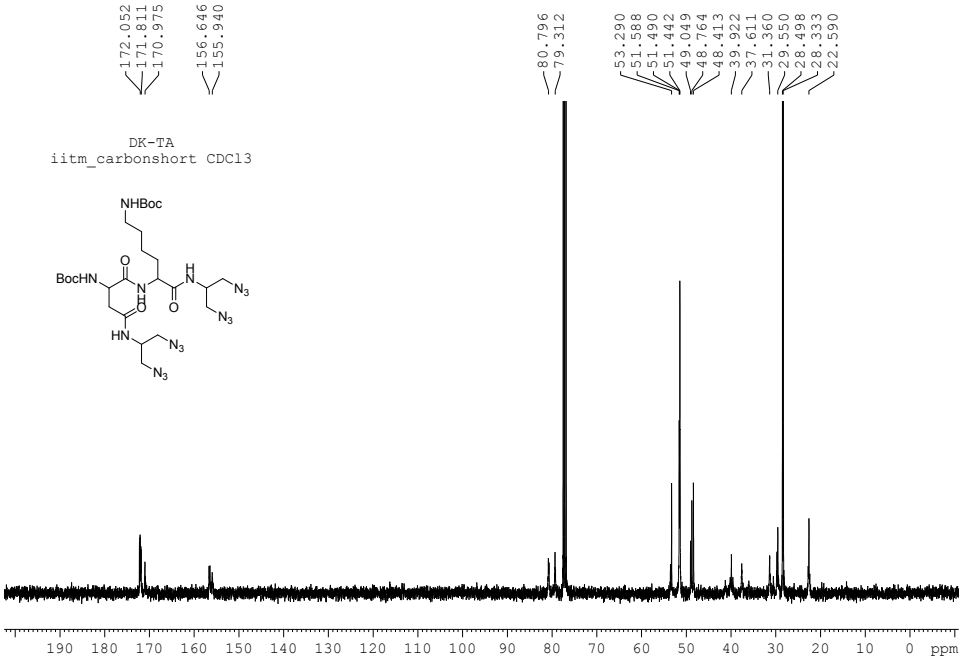
S76: ESI-MS spectrum of **26**.



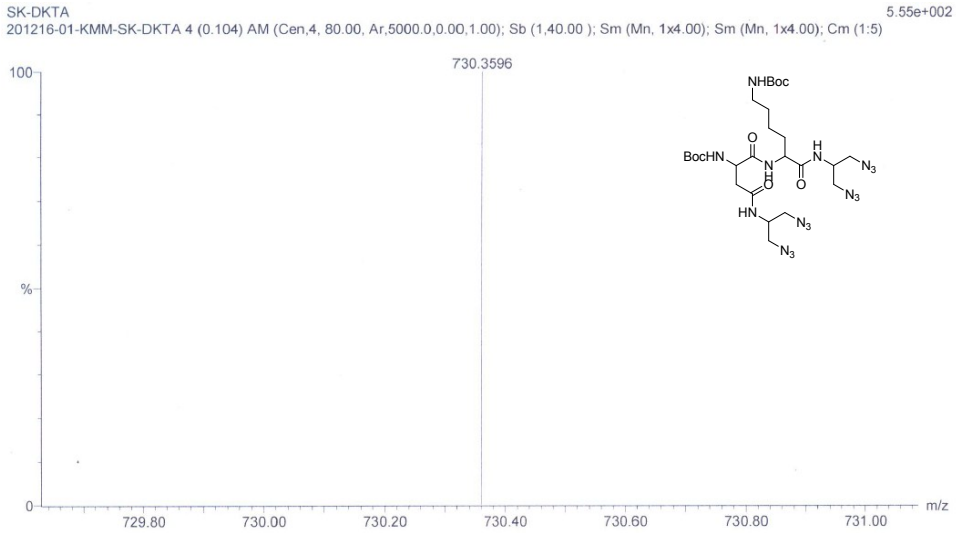
S77: isotopic pattern theoretical (above) and experimental (below) corresponding to $[M+K]^+$ of **26**.



S78: ^{1}H NMR spectrum of tetraazide **31**. (400 MHz, $CDCl_3$)

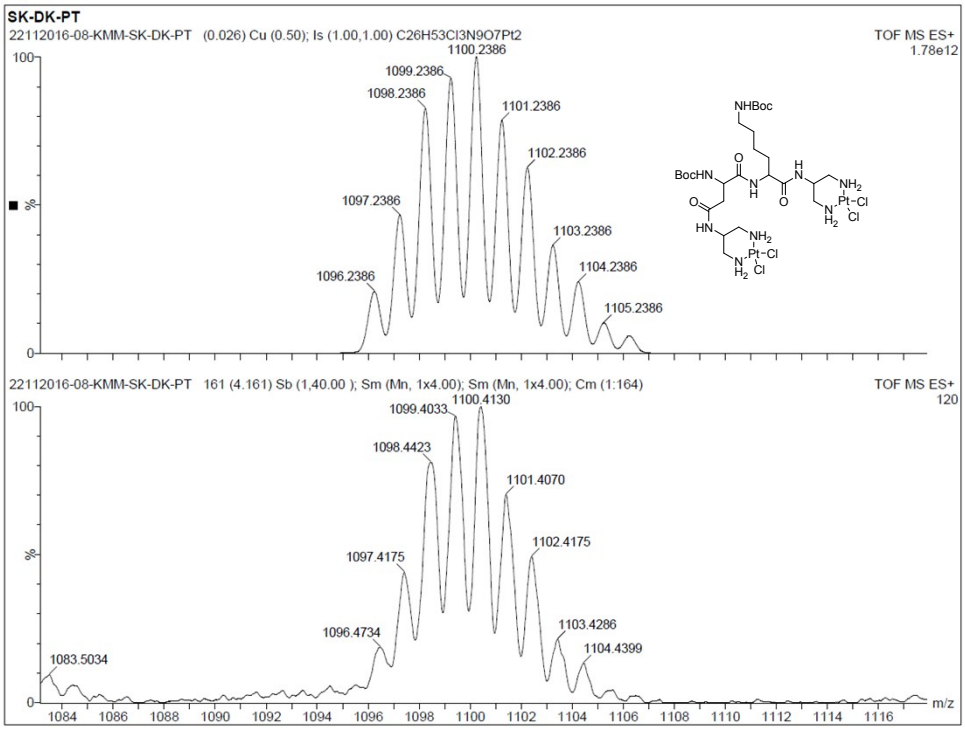


S79: ^{13}C NMR spectrum of tetraazide **31**. (100 MHz, $CDCl_3$).

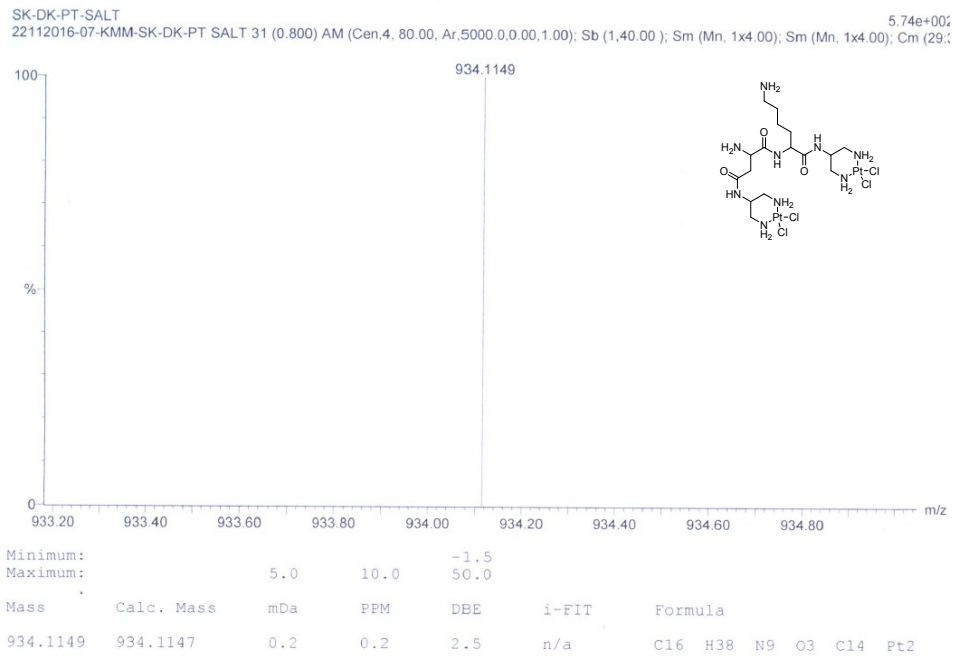


Minimum:				−1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
730.3596	730.3586	1.0	1.4	12.5	n/a	C26 H45 N17 O7 Na

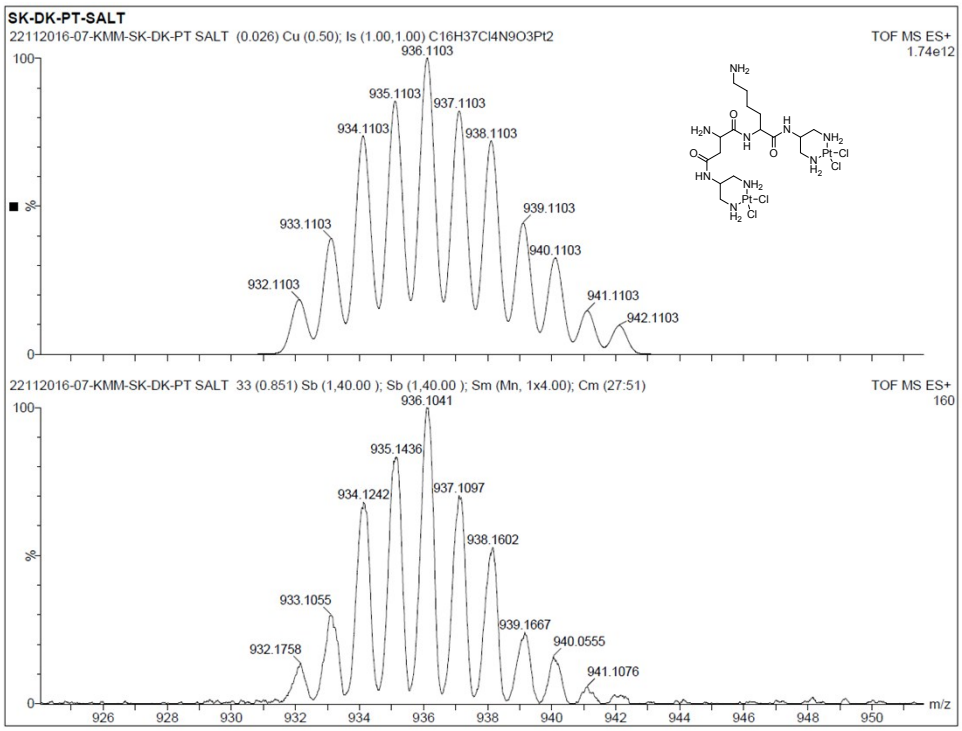
S80: HRMS of tetraazide **31**.



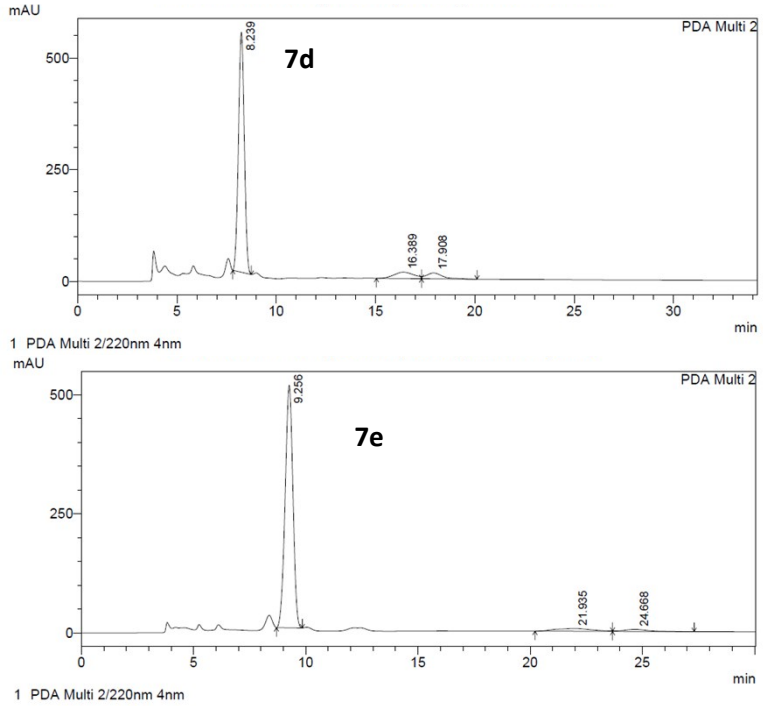
S81: isotopic pattern of **32a** corresponding to $[M\text{-}Cl]^+$ theoretical (above) and experimental (below).



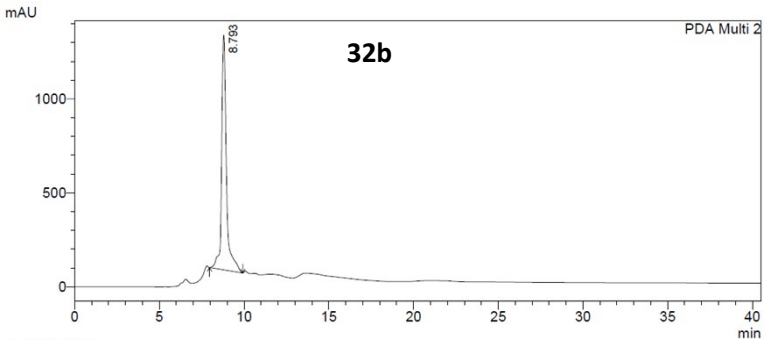
S82: HR-MS of **32b** corresponding to $[M\text{-}Cl]^+$.



S83: isotopic pattern of **32b** corresponding to $[M+H]^+$ theoretical (above) and experimental (below).



S84: HPLC-chromatograms of Pt-conjugates **7e**, **7d**. Analysis performed using analytical reverse phase C18 column (100A, 250x4.60 mm, 5 micron) using isochratic mobile phase of water/Acetonitrile (with 0.1%TFA) (1:10) with a flow rate of 0.5 mL/min; for analysis the sample**7d** and **7e** were dissolved in Acetonitrile. data acquired at 220nm of detector wavelength.

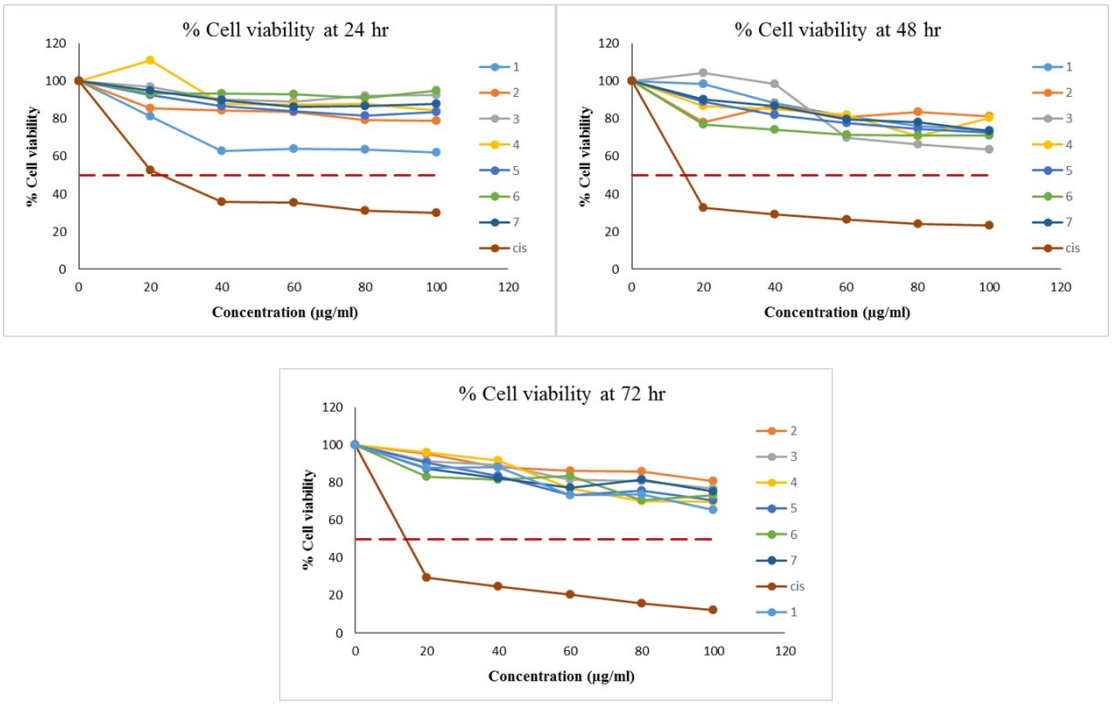


S85: HPLC-chromatogram of diPt-conjugate **32b**. Isochratic mobile phase of water/Acetonitrile (with 0.1%TFA) (3:1) was used; sample dissolved in water. Flow rate 0.5 mL/min. data acquired at 220nm of detector wavelength

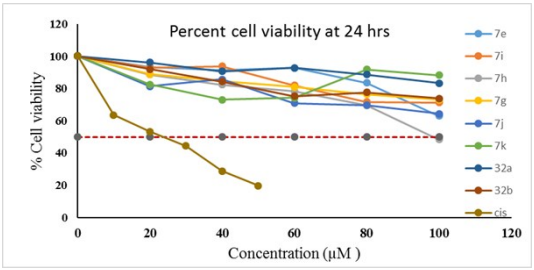
Table S1:[a] IC_{50} values of peptide Pt conjugates (after 48 hours of incubation)

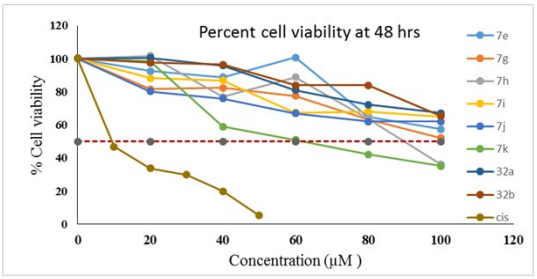
SL No.	Compound	IC_{50} ± Std.dev	SL No.	Compound	IC_{50}± Std.dev
1	7b	116.51±0.0017	**11**	7h	172.53±0.0108
3	7c	112.54±0.0018	**12**	7i	**44.52±0.0101**
4	4b	80.46±0.0038	**13**	7j	116.85±0.0063
5	4a	77.65±0.0041	**14**	7k	**51.11±0.0028**
6	7a	99.72±0.0085	**15**	32a	**75.91±0.0046**
7	7e	80.72±0.0110	**16**	32b	**83.40±0.0062**
8	7d	104.79±0.0131	**17**	19	255.86±0.0038
9	7f	184.69±0.0121	**18**	21	126.54±0.0093
10	7g	83.16±0.0144	**19**	**Cisplatin**	25.31±0.0041

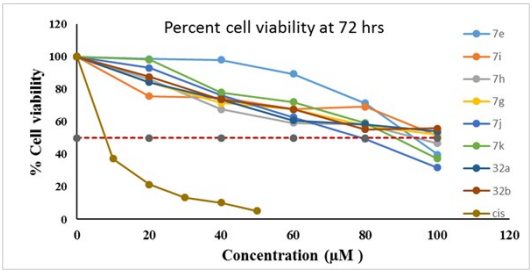
[a] Alamar blue assay was used for estimating cell viability in SiHa cells



S86: Cytotoxicity of free ligands compared with cisplatin; diamine precursors of **4a** (1), **7d** (2), **7e** (3), **32a** (4), **7f** (5), **7g** (6), **32b** (7).


Percent cell viability at 24 hrs
% Cell viability
Concentration (µM)
7e
7i
7h
7g
7j
7k
32a
32b
cis

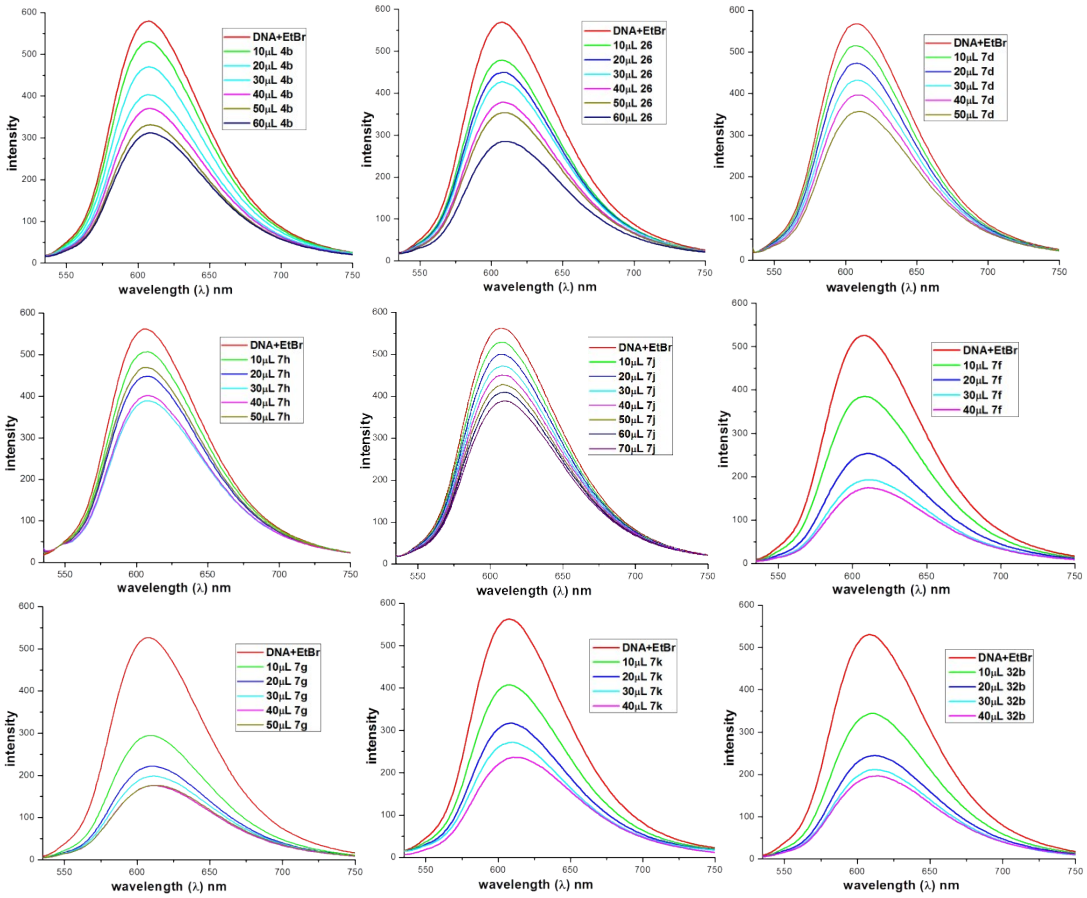

Percent cell viability at 48 hrs
% Cell viability
Concentration (µM)
7e
7g
7h
7i
7j
7k
32a
32b
cis


Percent cell viability at 72 hrs
% Cell viability
Concentration (µM)
7e
7i
7h
7g
7j
7k
32a
32b
cis

S87 : Cytotoxicity of peptide platinum conjugates against non-cancerous mouse fibroblastic cell lines (NiH3t3) at different incubation times (cell viability assessed by alamar blue assay).

Table S2: Therapeutic index values of the peptide platinum conjugates.

Compund code	**IC_{50} (µM) 3t3 cells**	**IC_{50} (µM) SiHa cells**	**T.I (IC_{50} Normal cells/ IC_{50} cancer cells)**
7k	69.85786	51.1	1.36
7i	111.3236	44.5	2.50
7g	130.0658	98.1	1.33
7j	117.9429	116.85	1.00
7e	134.2073	80.72	1.66
32a	145.7825	75.91	1.92
32b	167.0894	83.40	2.00
cisplatin	18.267	25.31	1.38



S88: Ethidium bromide displacement assay results.