SUPPLEMENTARY INFORMATION

for

A tri-functional vanadium(IV) complex to detect cysteine oxidation

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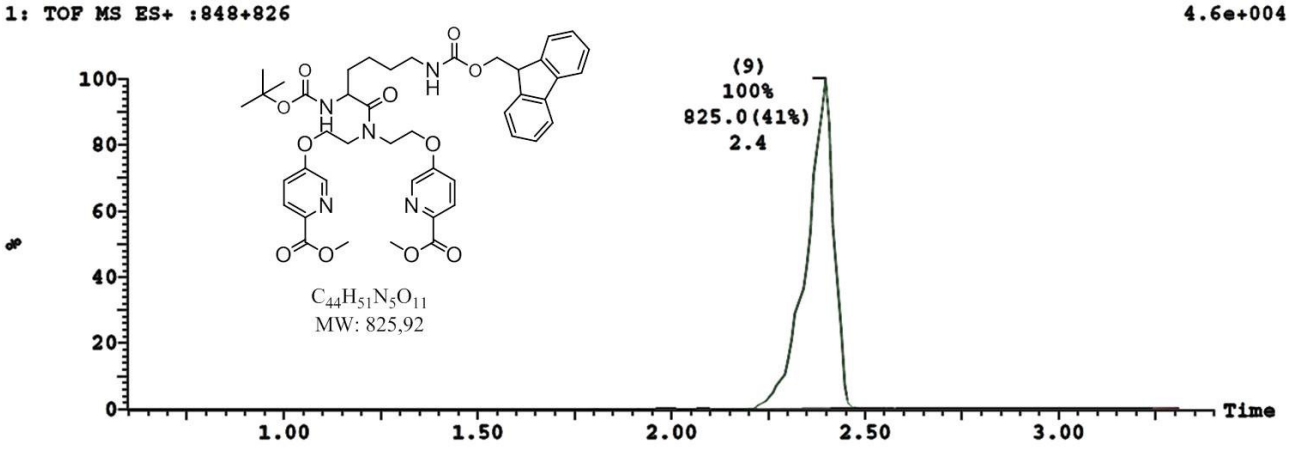
Content (spectra and chromatograms):

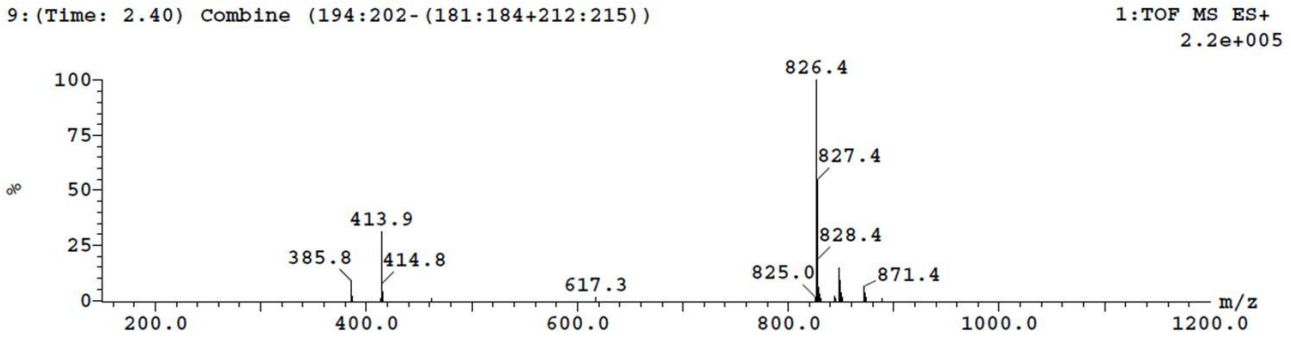
Captions:

1. LC-MS and ^{1}H NMR for dipicolinic intermediates 2-6 and 9-12

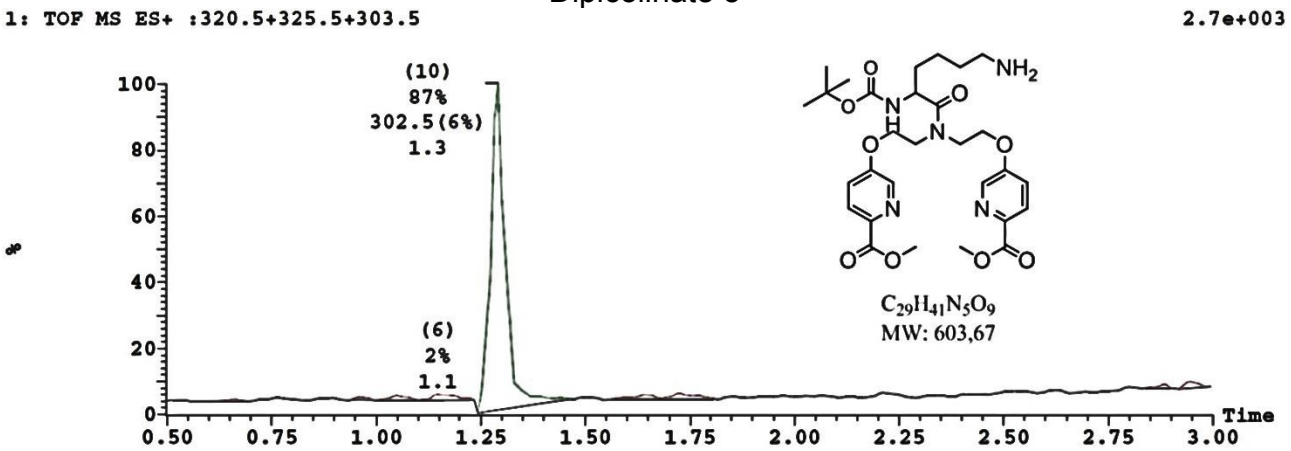
Dipicolinate **2**



Peak ID	Compound	Time	Mass Found
9	Found	2.40	848.00,826.00



Dipicolinate **3**



Peak ID	Compound	Time	Mass Found
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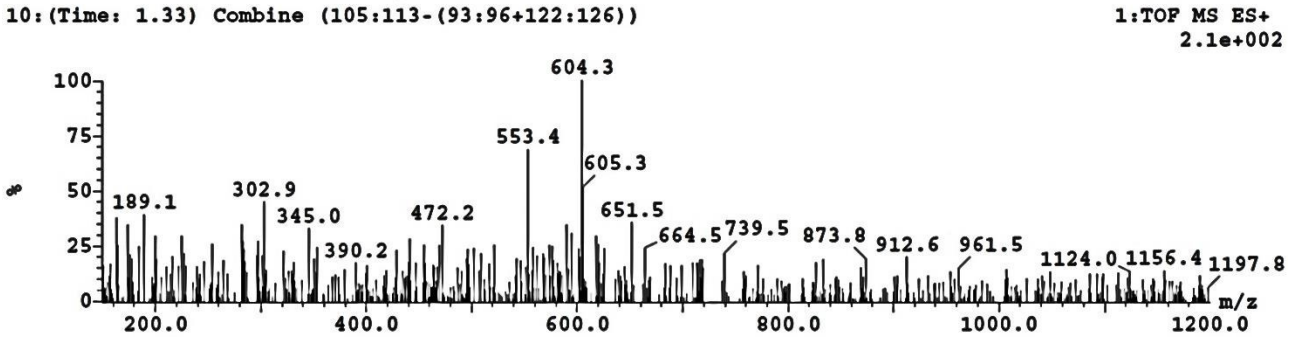
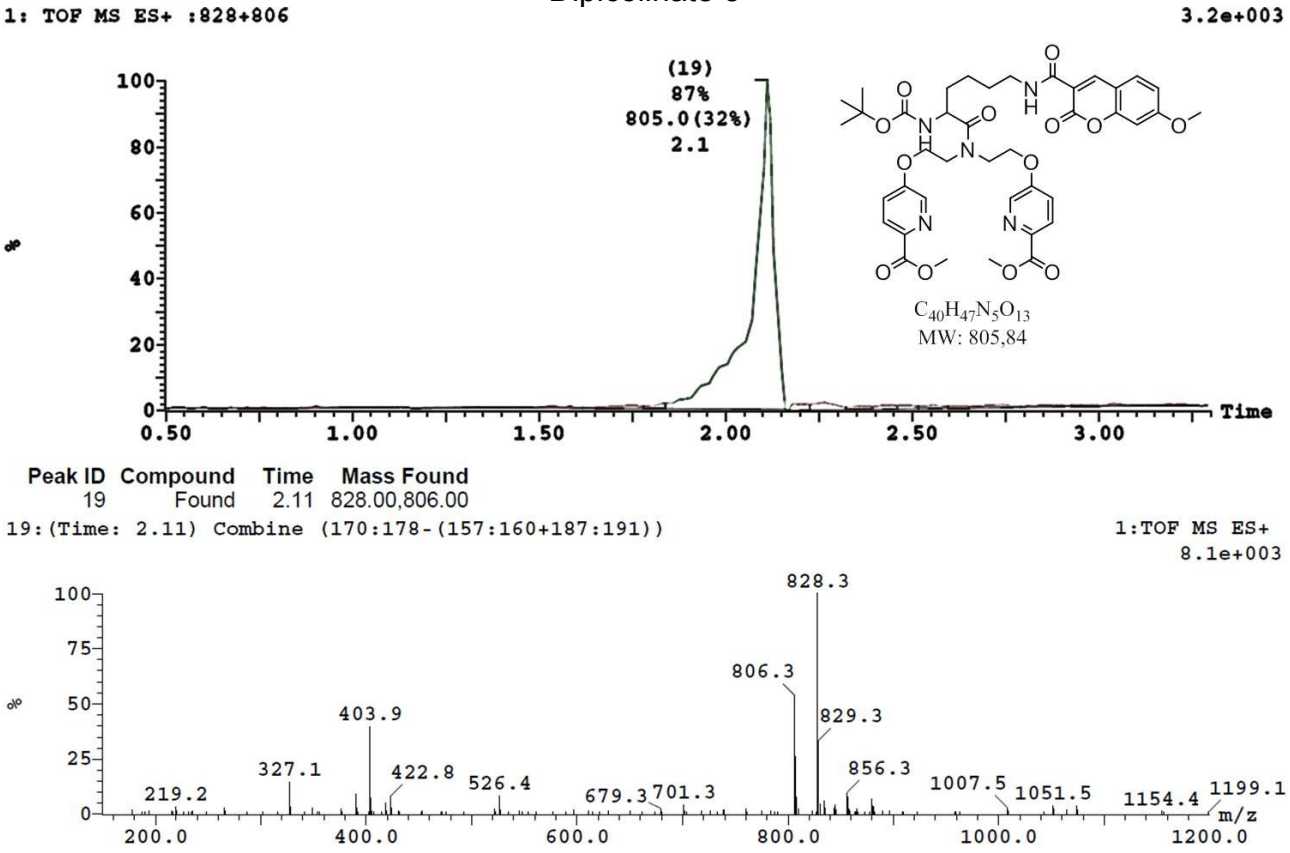


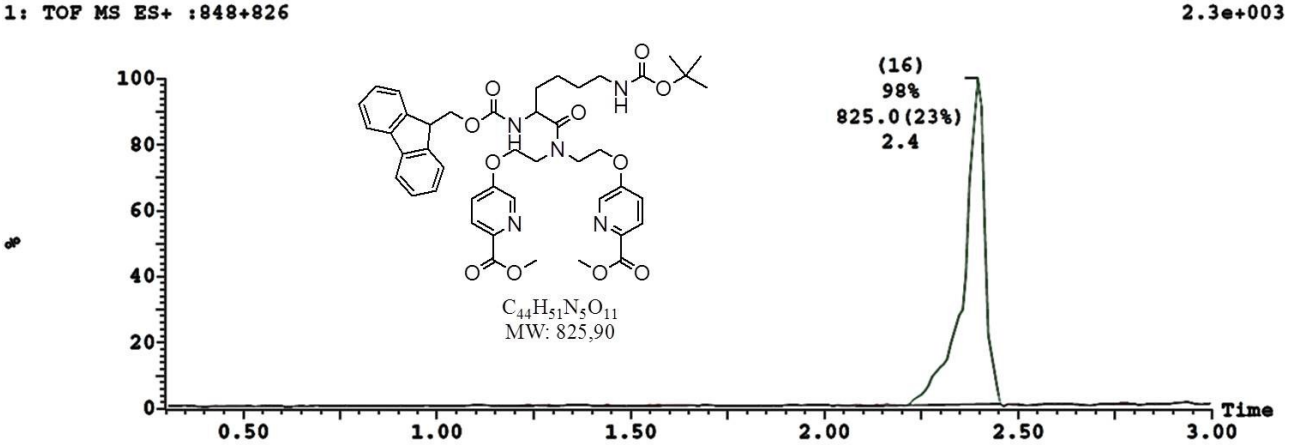
Dipicolinate **5**



Peak ID	Compound	Time	Mass Found
19	Found	2.11	828.00,806.00

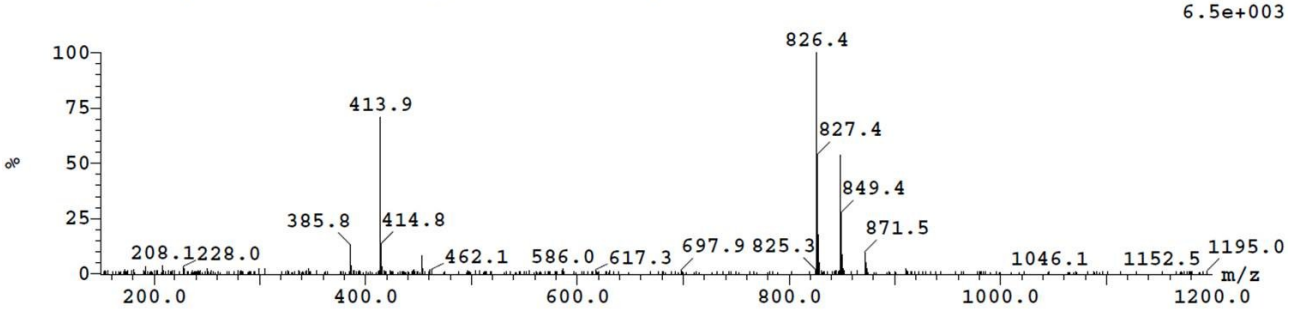
19:(Time: 2.11) Combine (170:178-(157:160+187:191))

Dipicolinate **9**

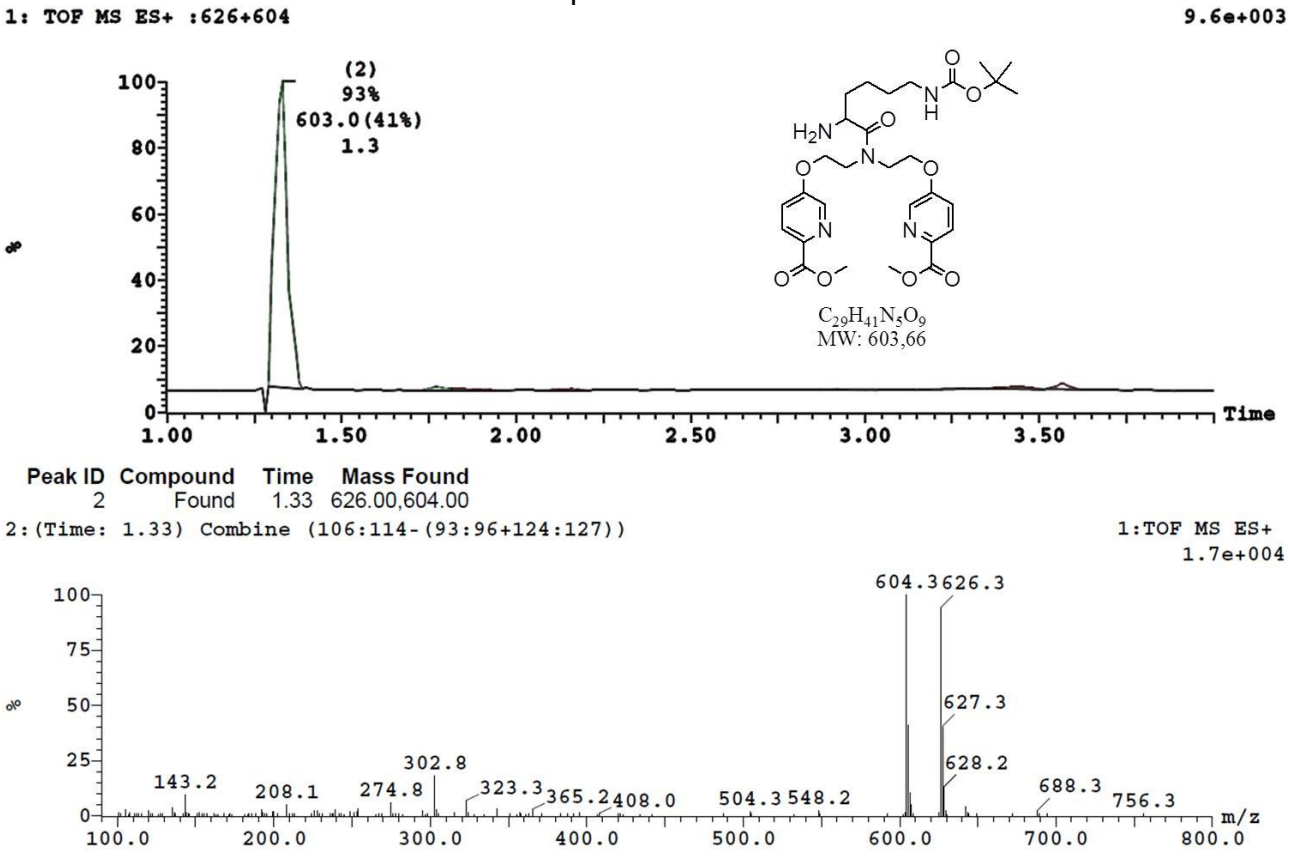


Peak ID	Compound	Time	Mass Found
16	Found	2.40	848.00,826.00

16:(Time: 2.40) Combine (193:201-(181:184+211:214))

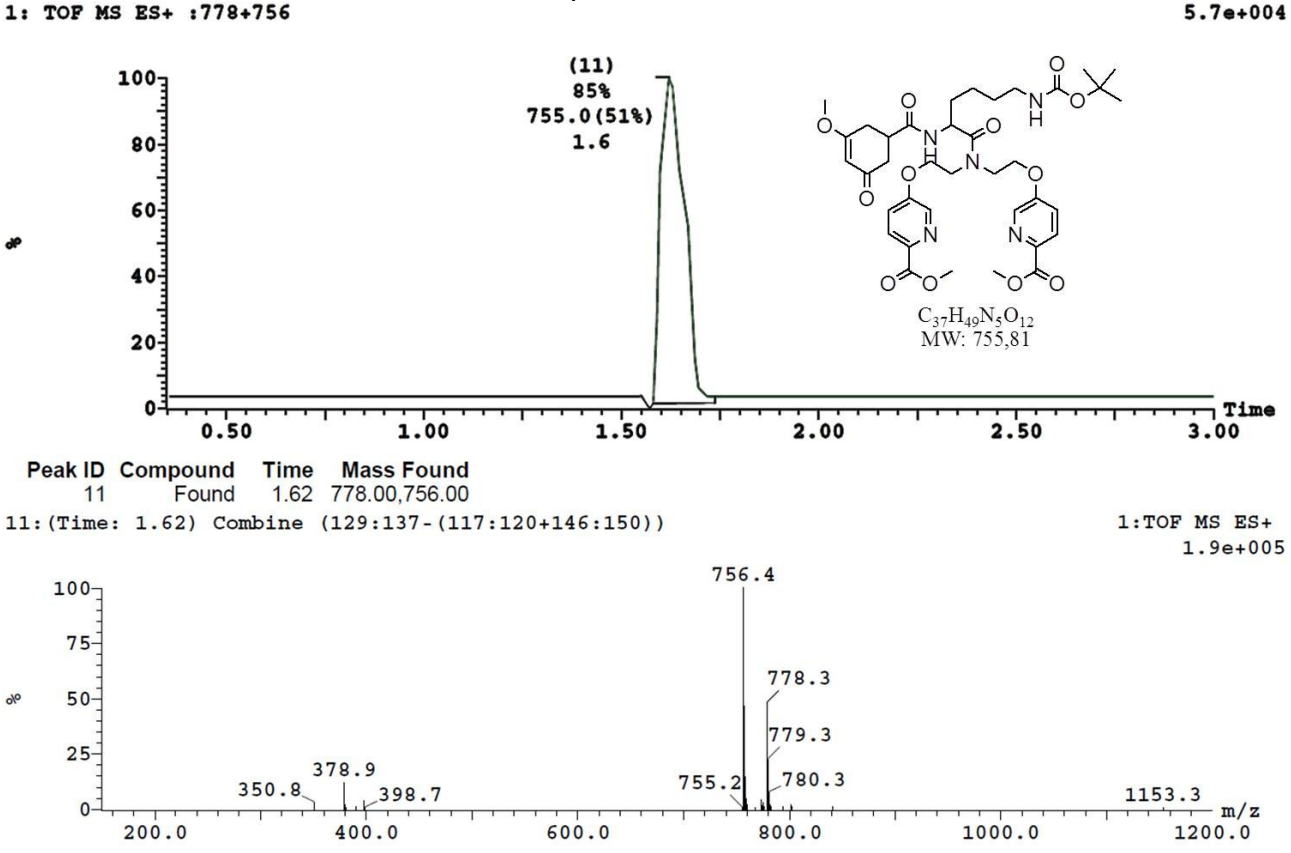


Dipicolinate **10**



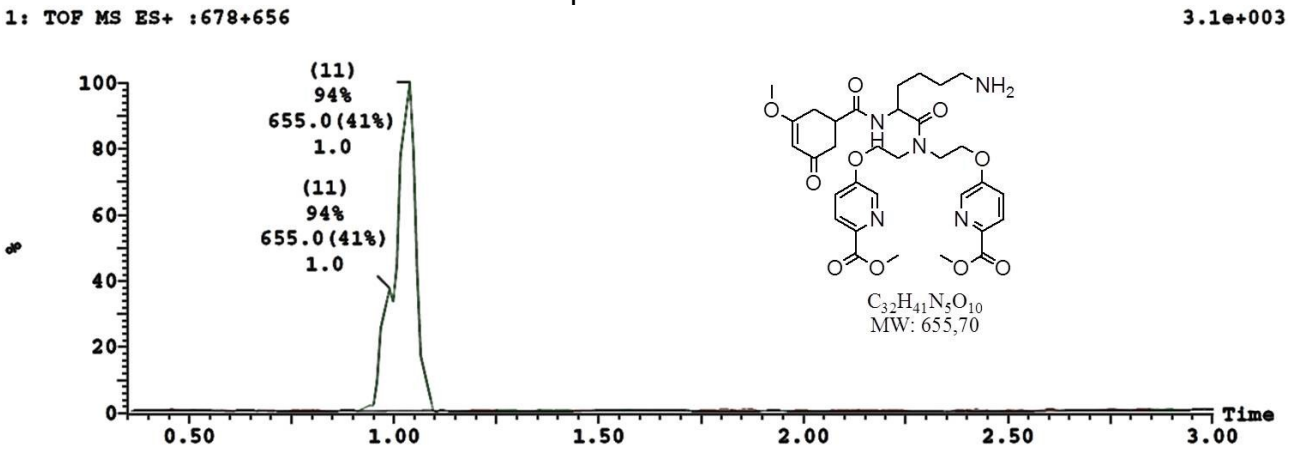
$C_{29}H_{41}N_5O_9$
MW: 603,66

Dipicolinate **11**



$C_{37}H_{49}N_5O_{12}$
MW: 755,81

Dipicolinate **12**



Peak ID	Compound	Time	Mass Found
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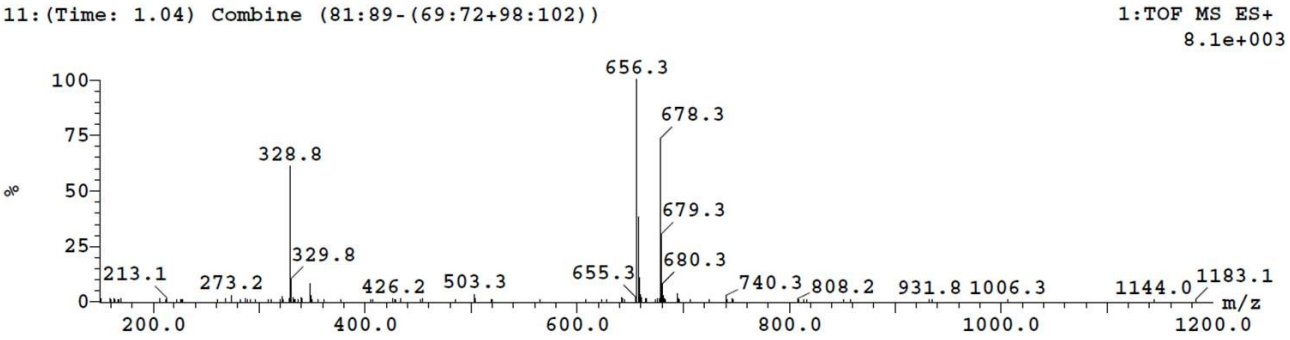
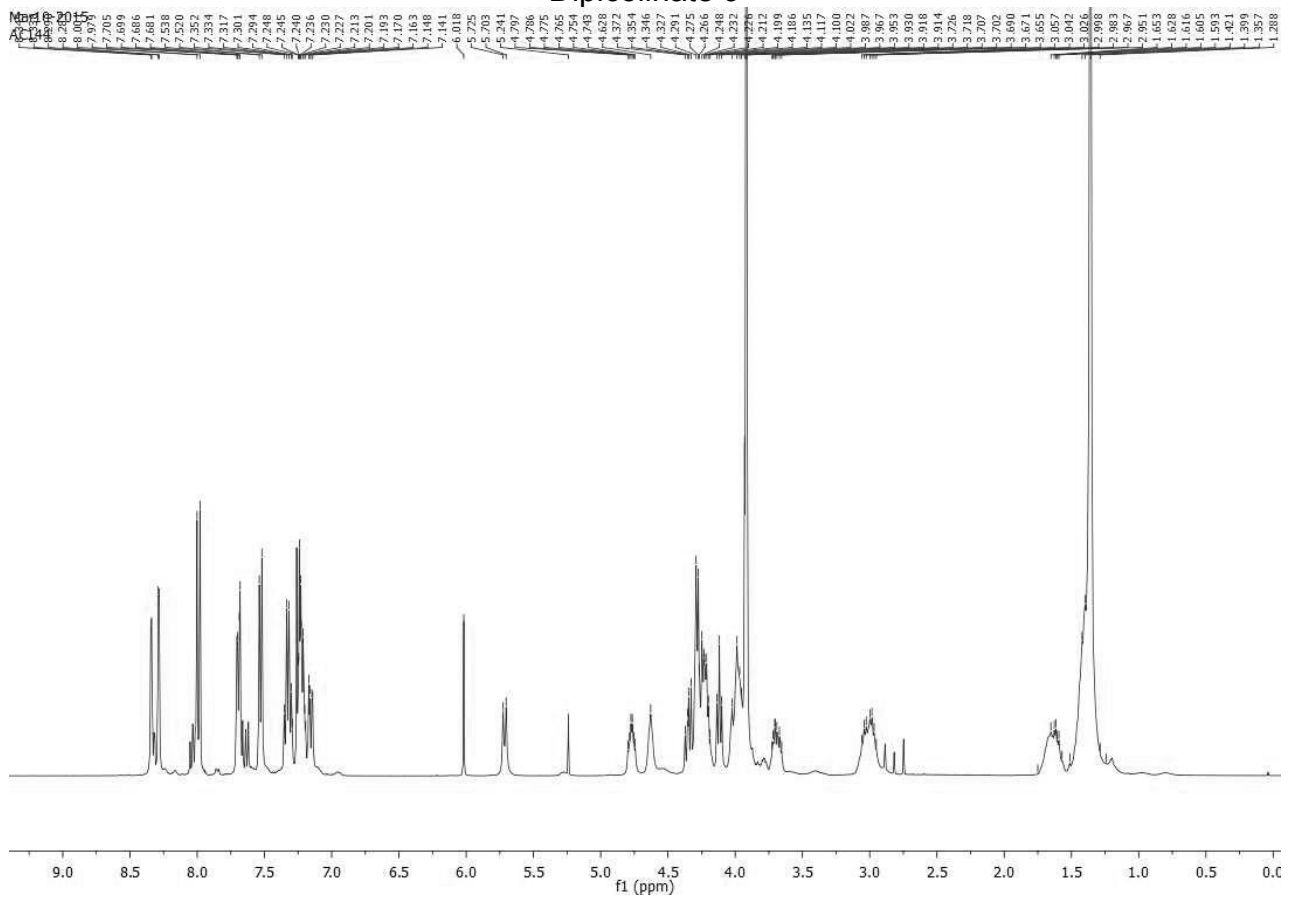
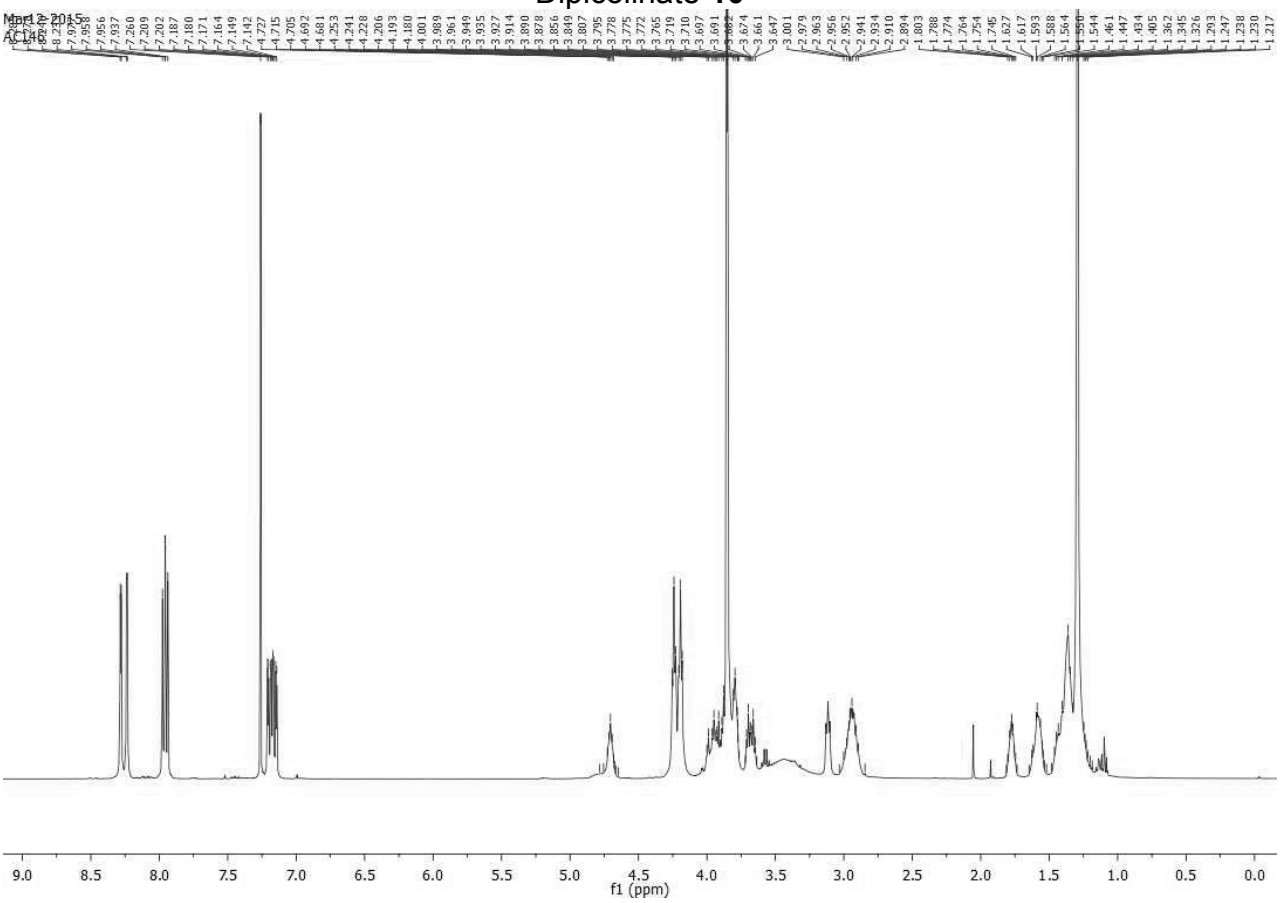


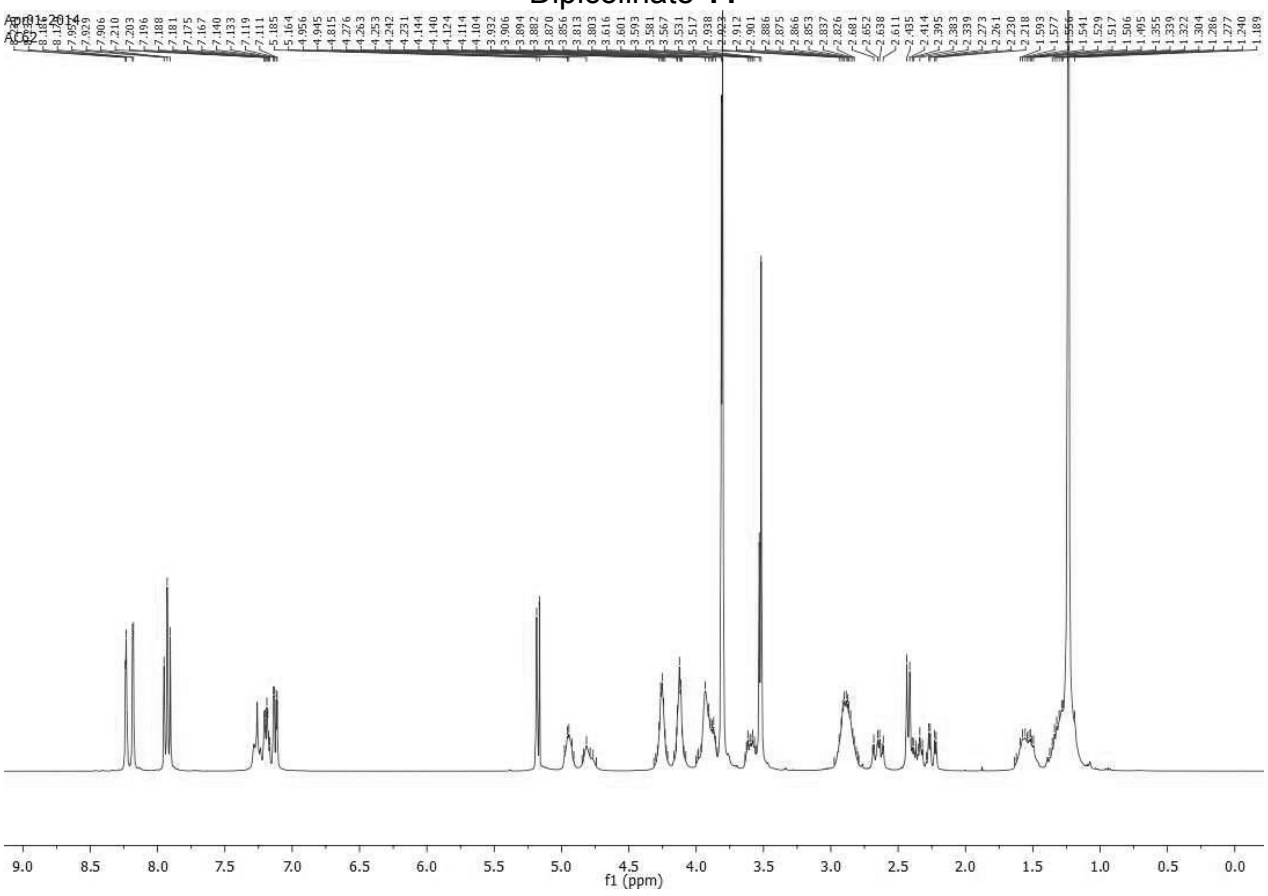
Dipicolinate **9**



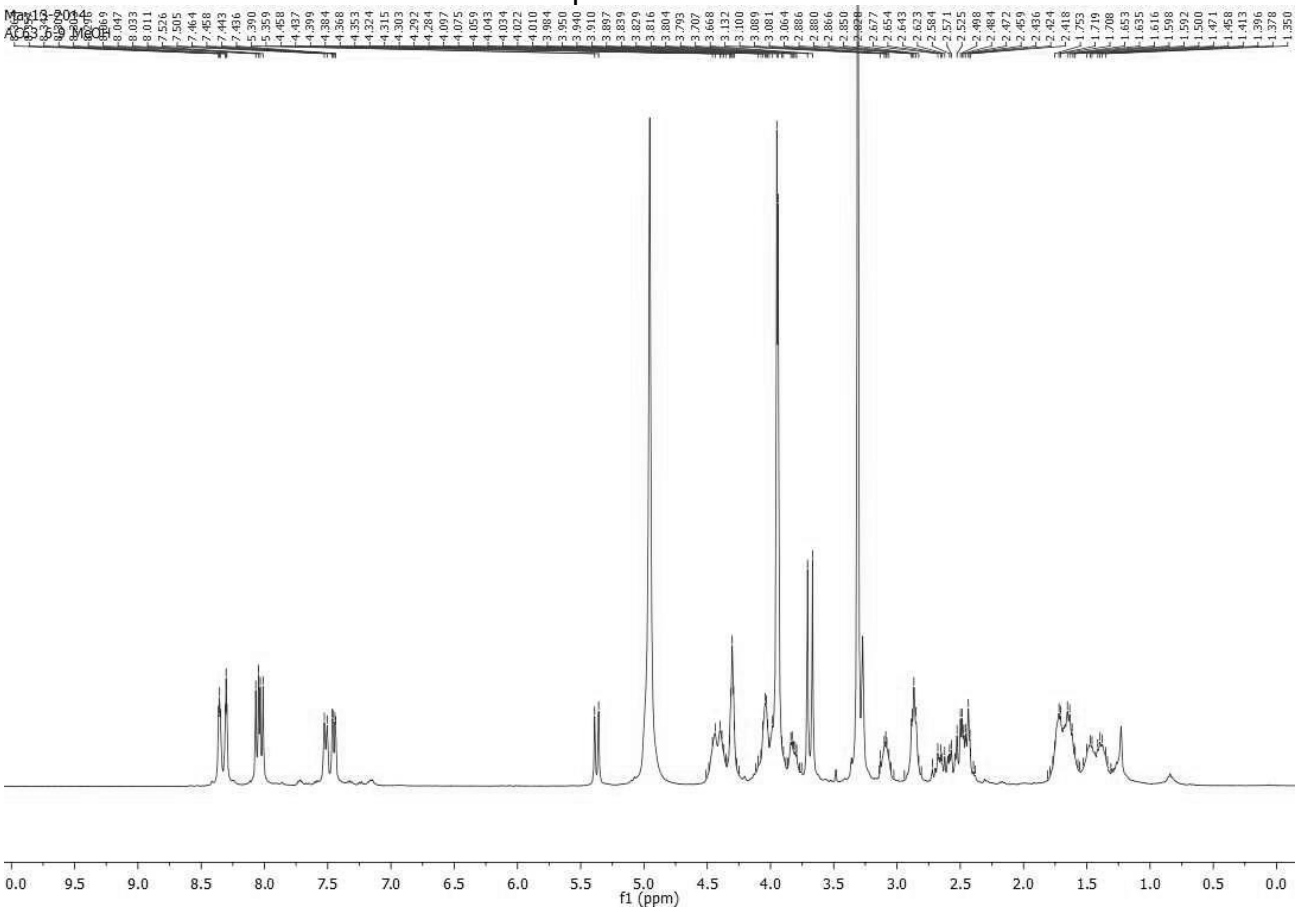
Dipicolinate **10**



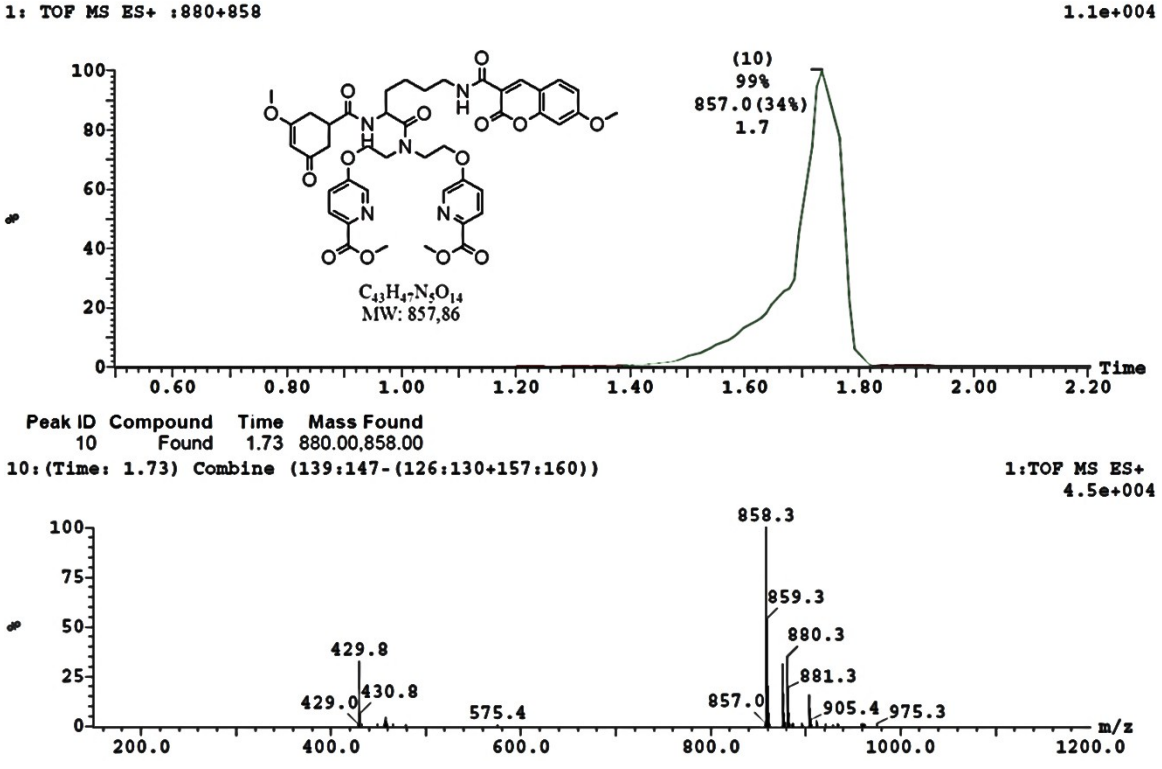
Dipicolinate **11**



Dipicolinate **12**



2. LC-MS, HRMS and ^{1}H NMR for fully protected dipicolinate 14



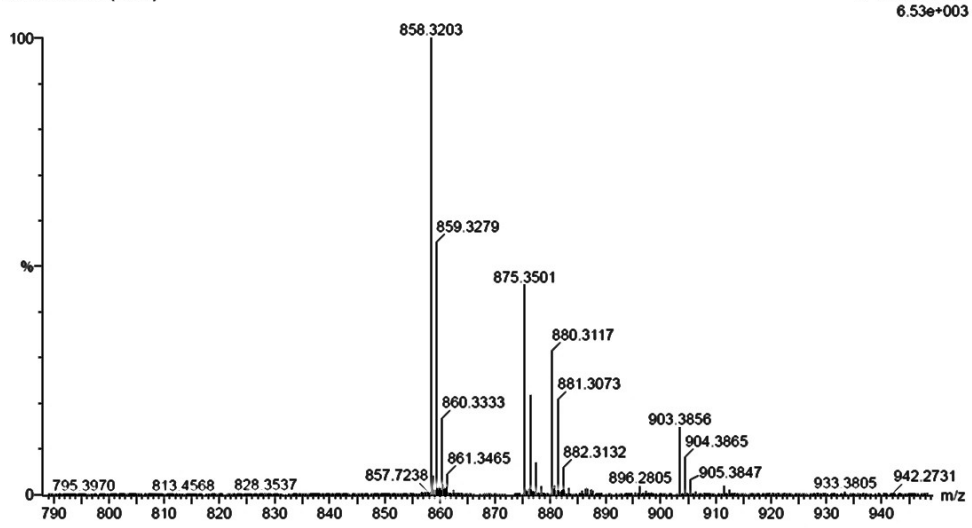
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

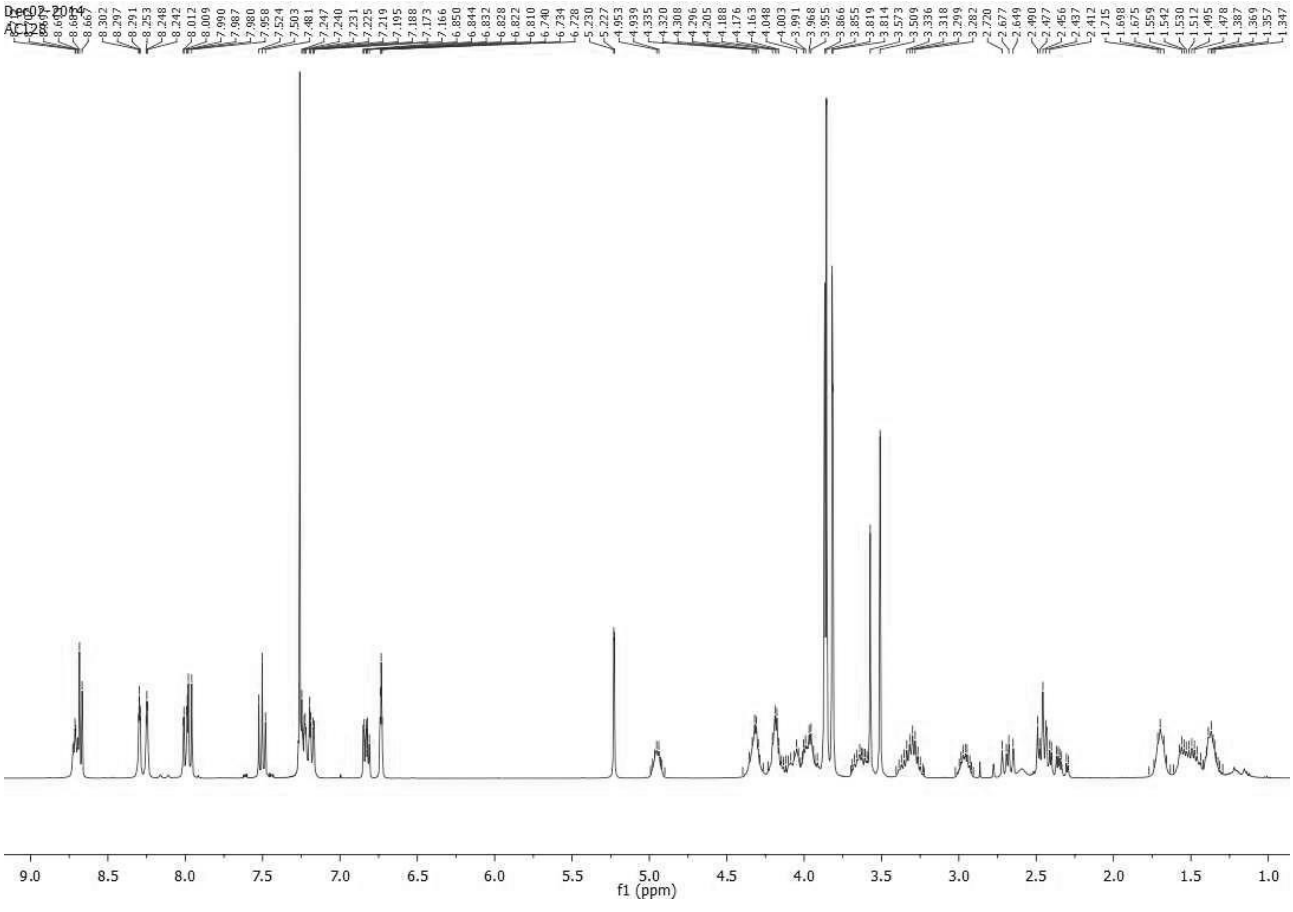
Monoisotopic Mass, Even Electron Ions
328 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 43-43 H: 0-200 N: 0-10 O: 0-14 Na: 0-1

A.CILIBRIZZI AC128
ms17122a 141 (1.717)

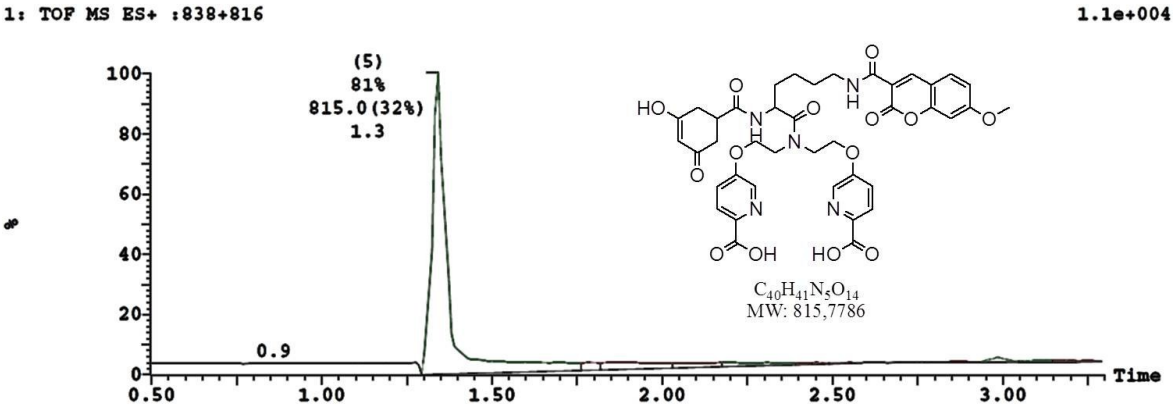
1: TOF MS ES+
6.53e+003



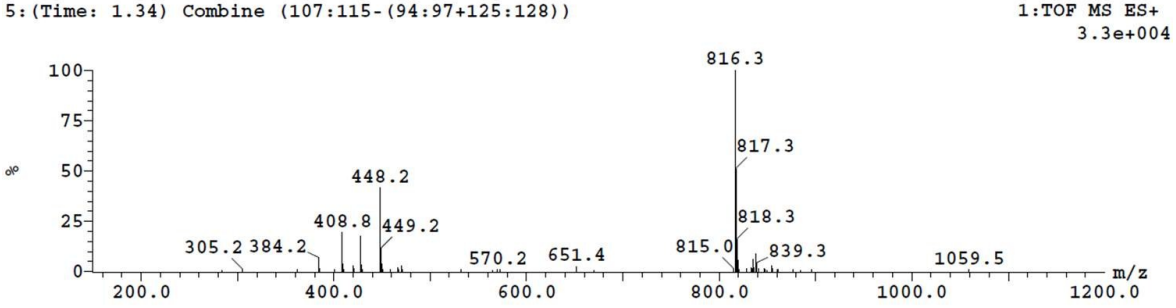
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**i-FIT**	**i-FIT (Norm)**	**Formula**
858.3203	858.3198	0.5	0.6	22.5	105.7	0.0	C43 H48 N5 O14



3. LC-MS, HRMS, $^{1}H/^{13}C$/COSY/HSQC NMRs, UV/vis and emission for ligand 15



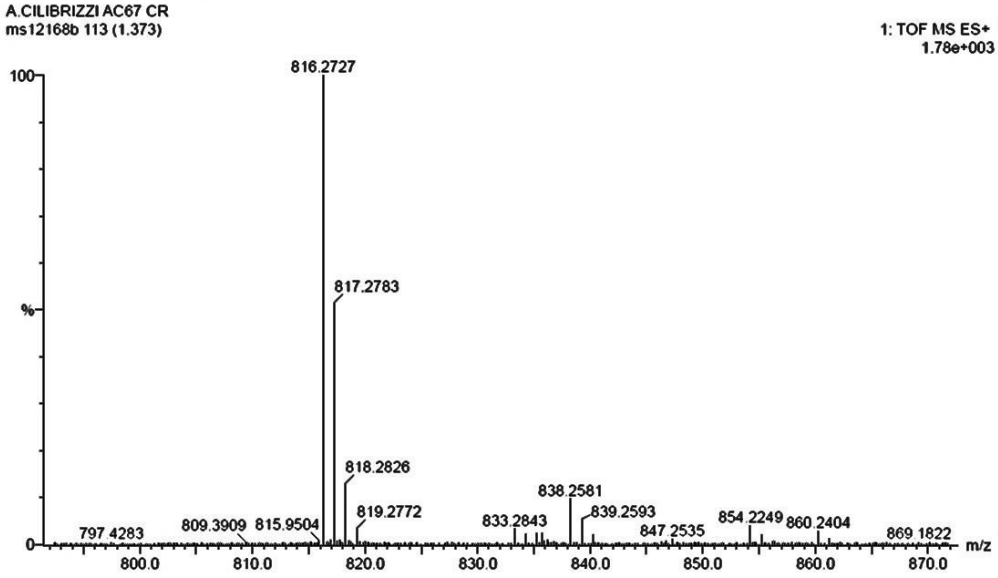
Peak ID	Compound	Time	Mass Found
5	Found	1.34	838.00,816.00



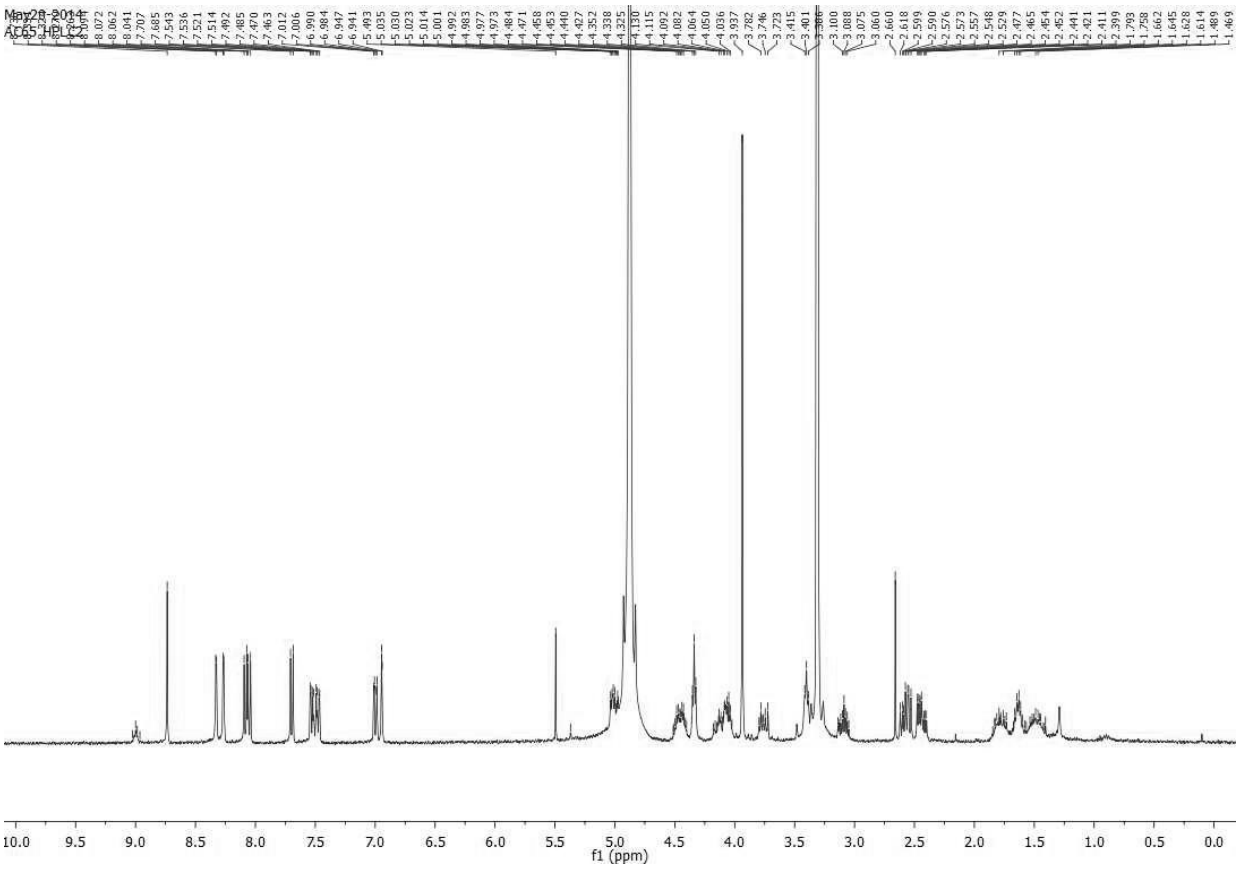
Elemental Composition Report **Page 1**

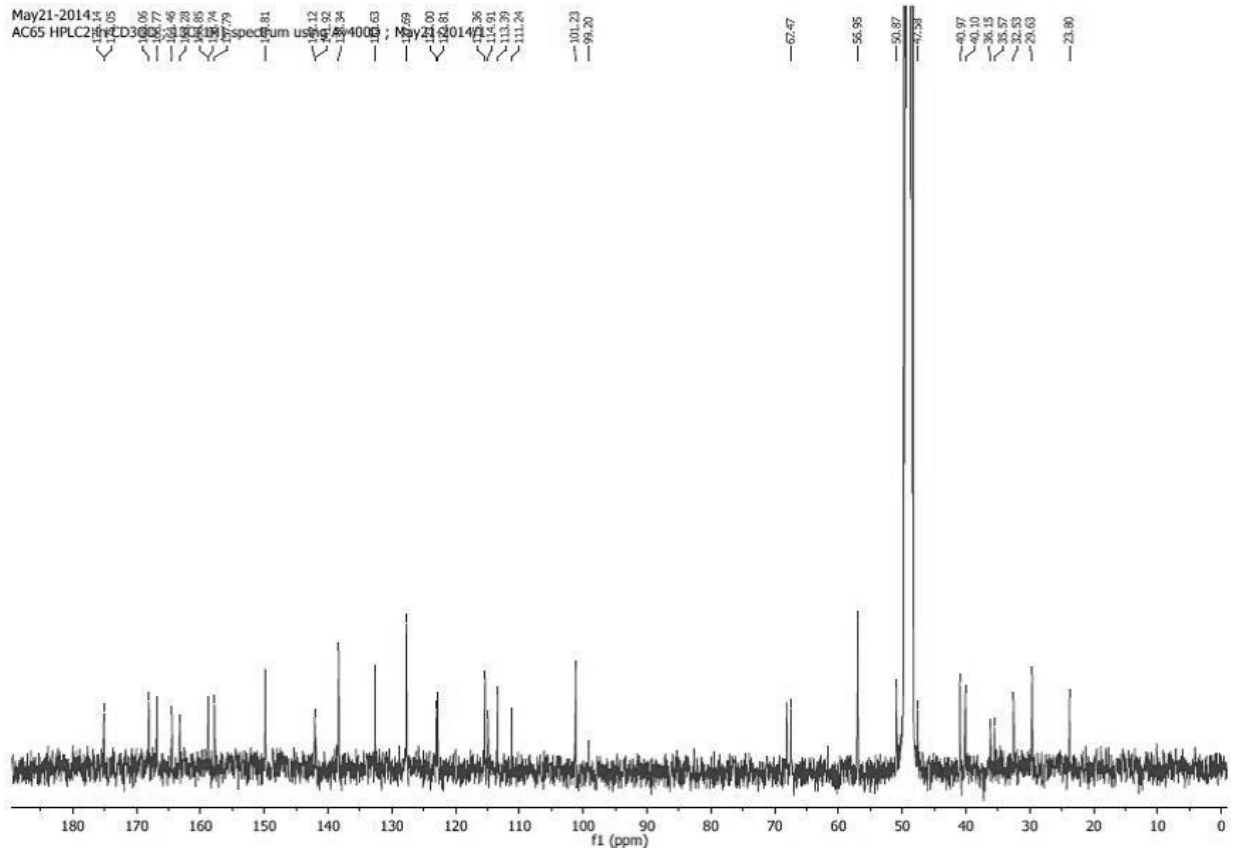
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

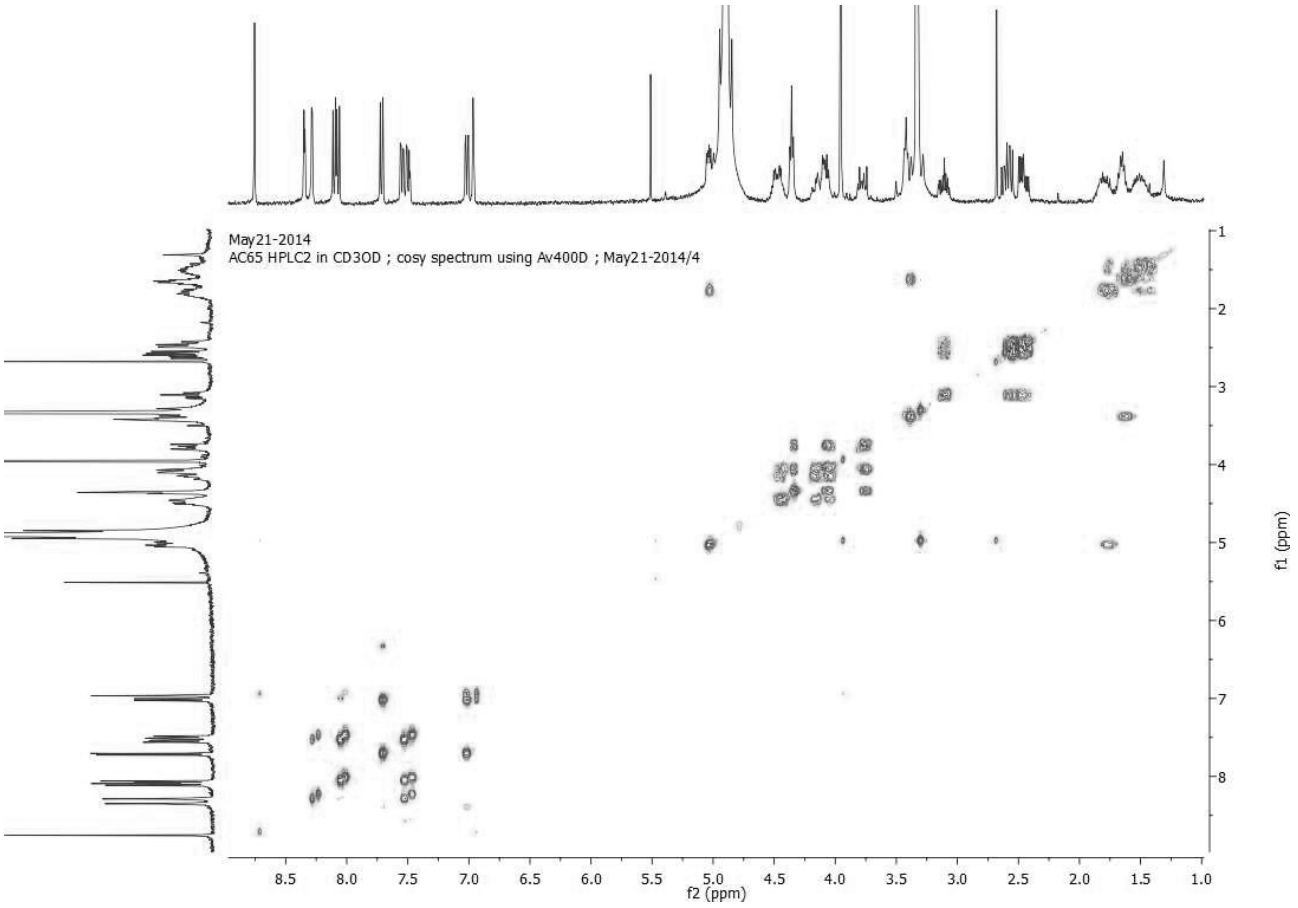
Monoisotopic Mass, Even Electron Ions
172 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 40-40 H: 5-300 N: 0-10 O: 0-15
A.CILIBRIZZI AC67 CR
ms12168b 113 (1.373)

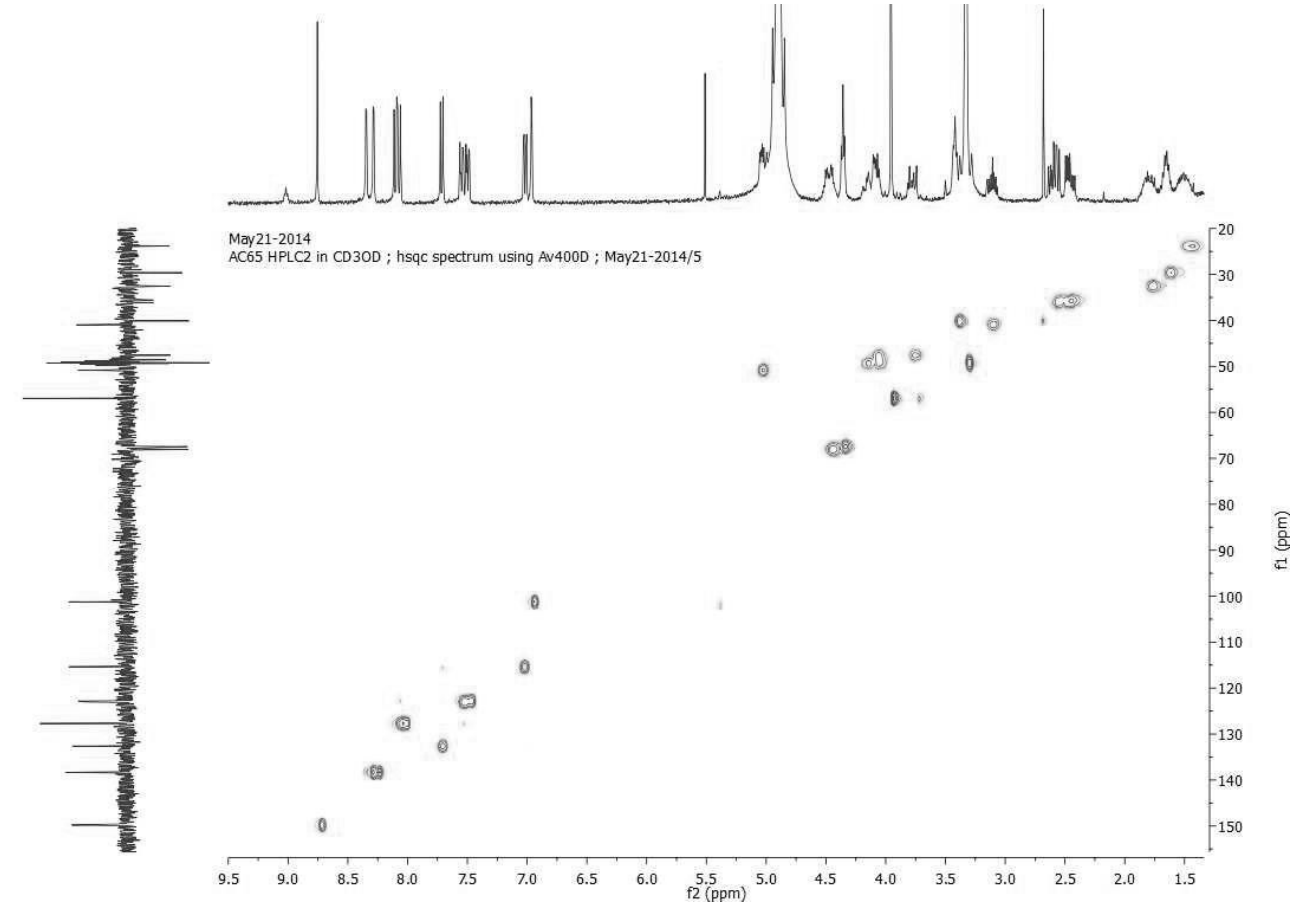


Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
816.2727	816.2728	-0.1	-0.1	22.5	55.7	0.0	C40 H42 N5 O14


f1 (ppm)


May21-2014
f1 (ppm)


May21-2014
AC65 HPLC2 in CD3OD ; cosy spectrum using Av400D ; May21-2014/4
f2 (ppm)
f1 (ppm)


May21-2014
AC65 HPLC2 in CD3OD ; hsqc spectrum using Av400D ; May21-2014/5
f2 (ppm)
f1 (ppm)

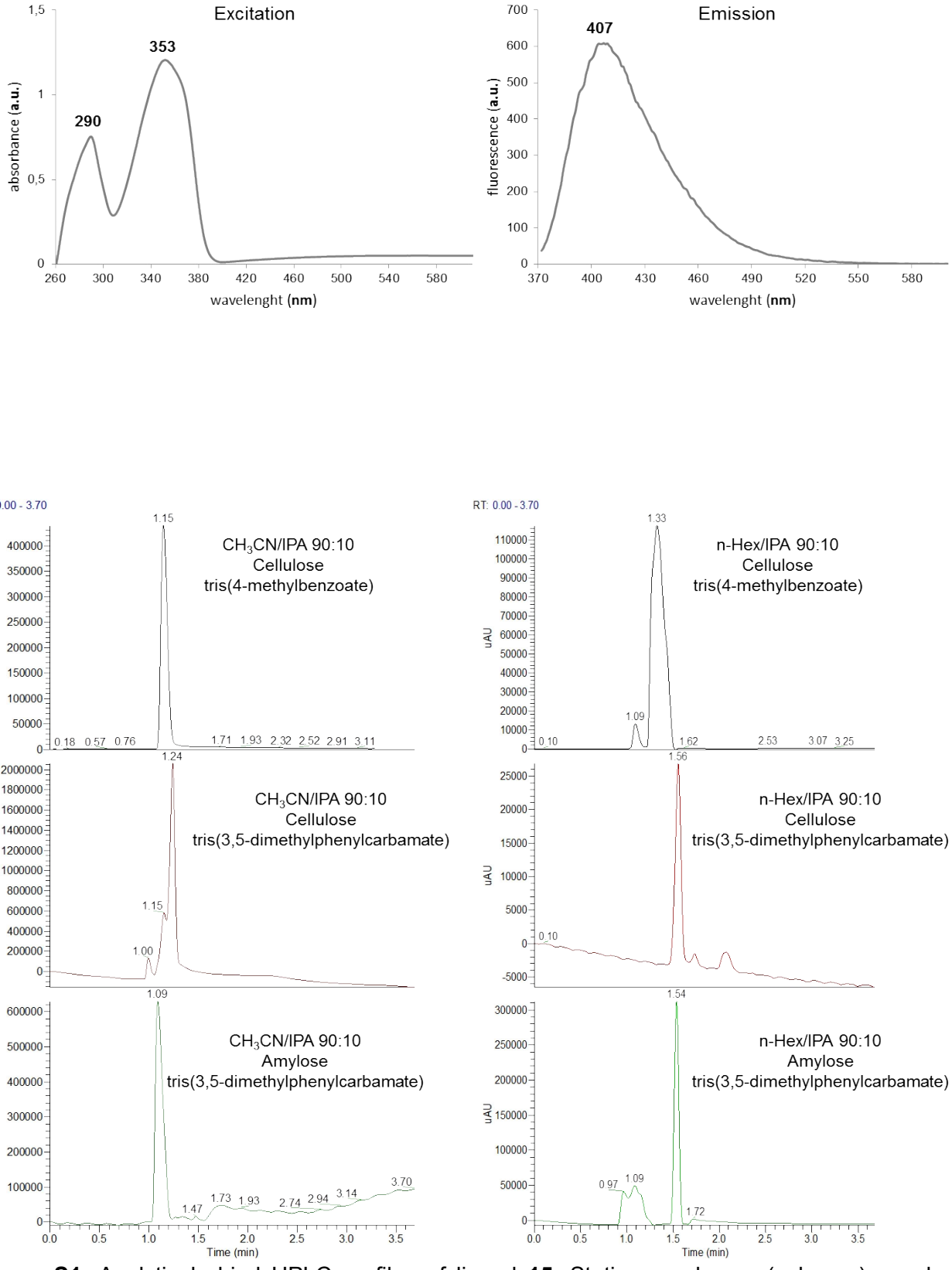
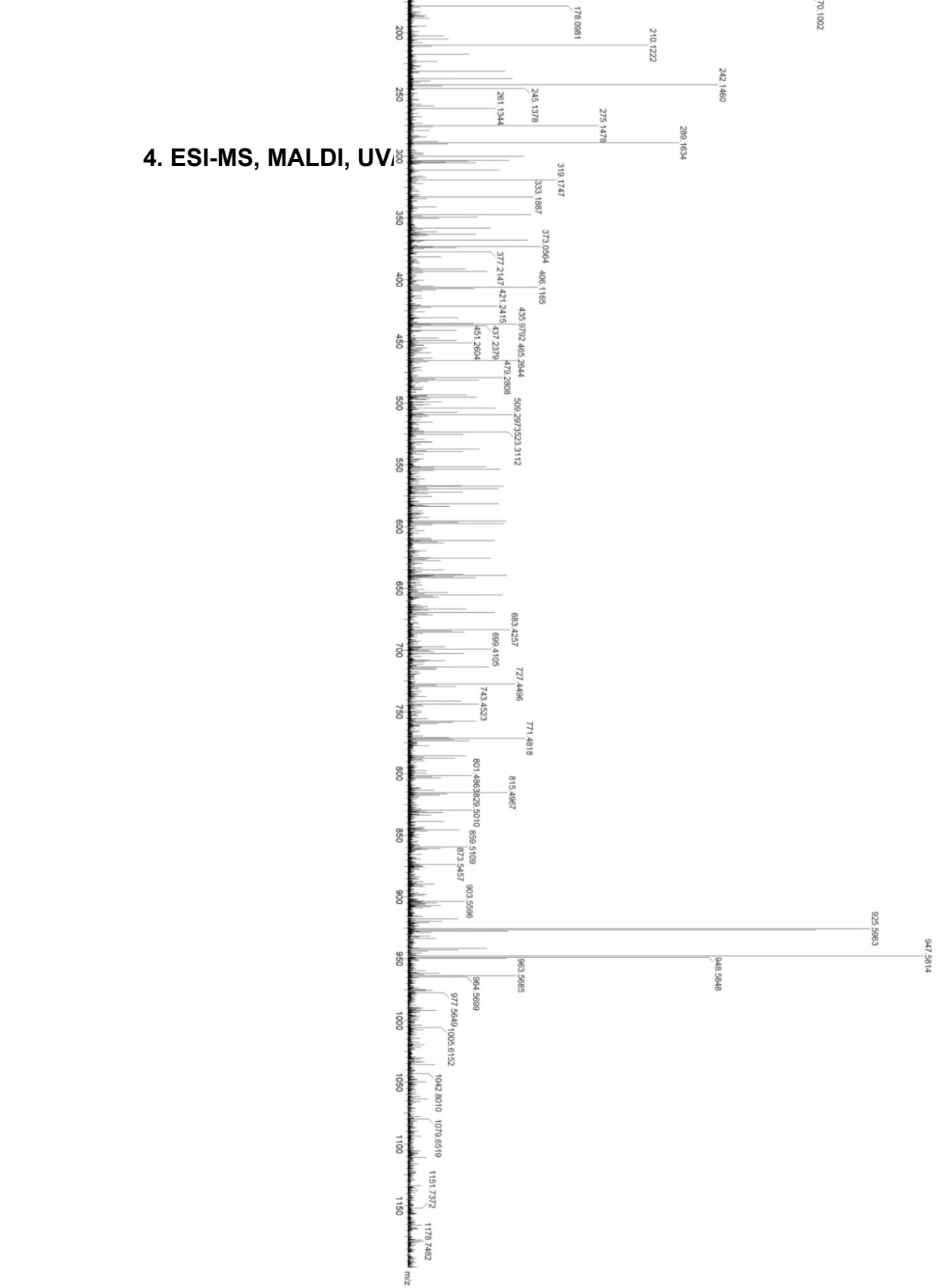
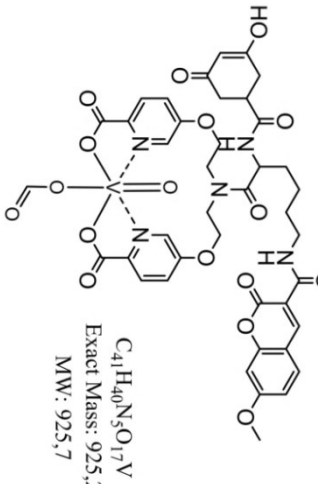
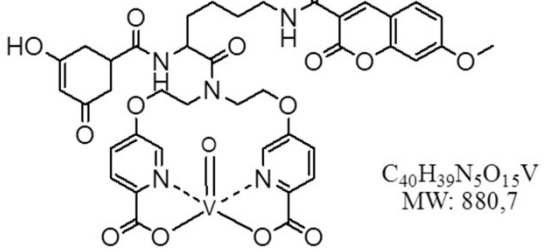


Figure S1. Analytical chiral HPLC profiles of ligand **15**. Stationary phases (columns): amylose tris(3,5-dimethylphenylcarbamate) (Lux Amylose-1), cellulose tris(3,5-dimethylphenylcarbamate) (Lux Cellulose-1), and cellulose tris(4-methylbenzoate) (Lux Cellulose-3); eluents: n-Hex/IPA and CH_3CN/IPA 90:10; flow rate: 1.0 mL/min; temperature: 25°C; UV detection: 254 nm.

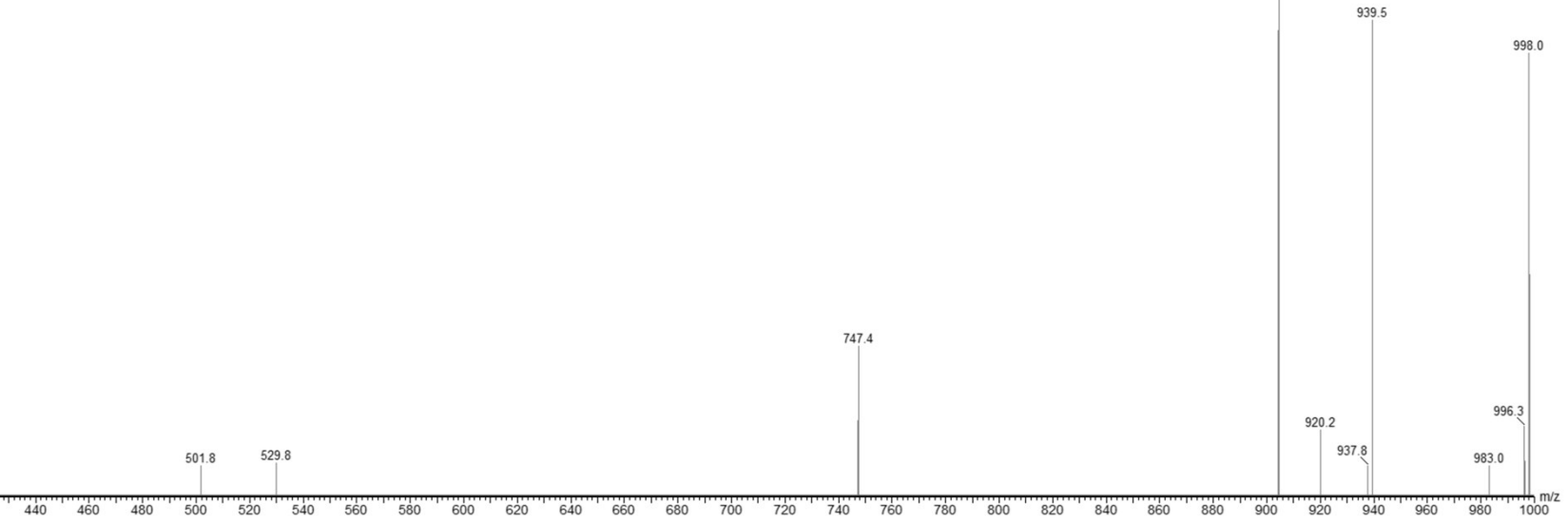
4. ESI-MS, MALDI, UV

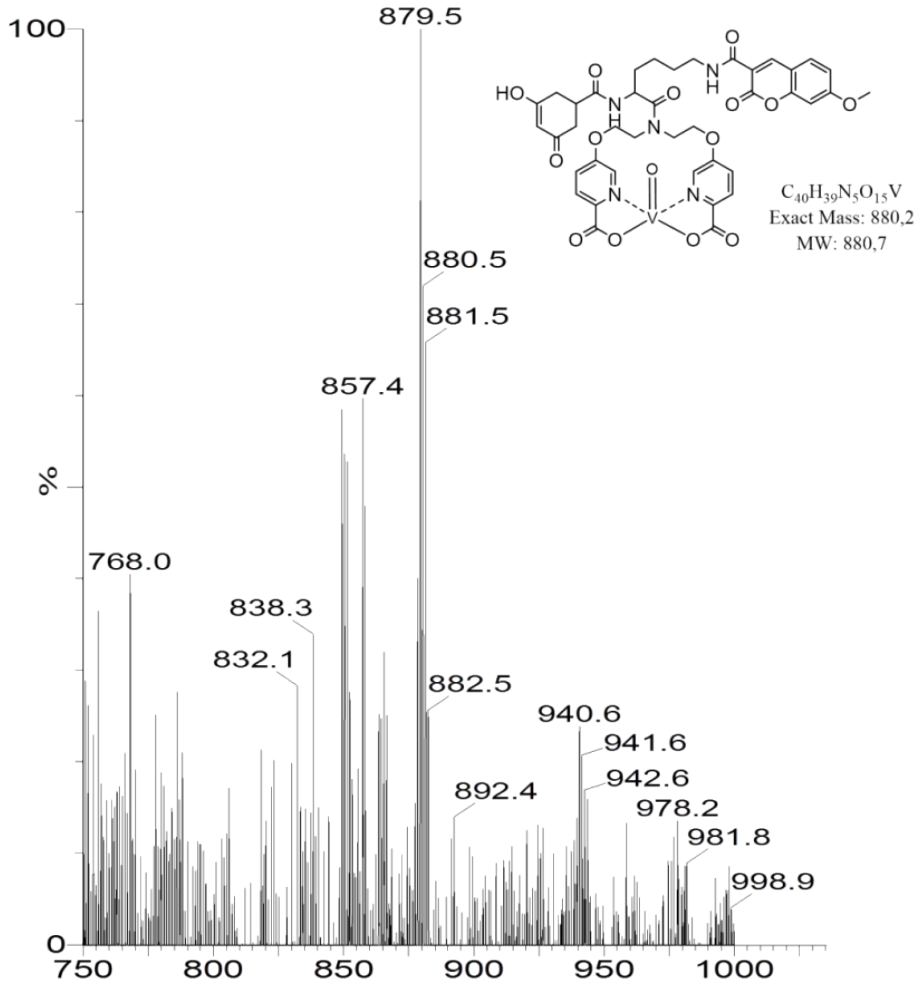


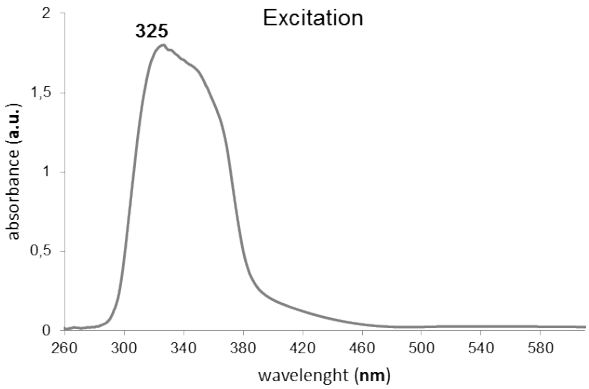


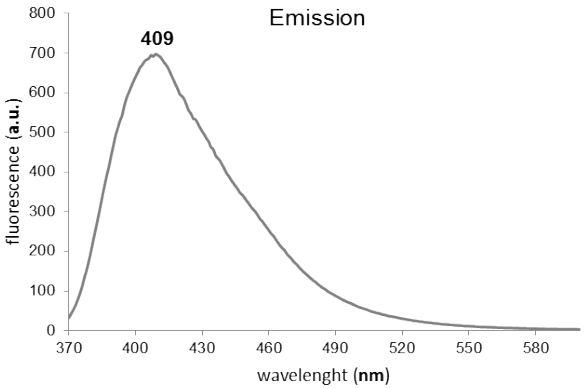


$C_{40}H_{39}N_5O_{15}V$
MW: 880,7




100
%
0
750
800
850
900
950
1000
879.5
880.5
881.5
857.4
768.0
838.3
832.1
882.5
940.6
941.6
942.6
892.4
978.2
981.8
998.9
HO
O
N
H
O
V
C40H39N5O15V
Exact Mass: 880,2
MW: 880,7


Excitation
325
absorbance (a.u.)
wavelenght (nm)
2
1,5
1
0,5
0
260
300
340
380
420
460
500
540
580


Emission
409
fluorescence (a.u.)
wavelenght (nm)
800
700
600
500
400
300
200
100
0
370
400
430
460
490
520
550
580

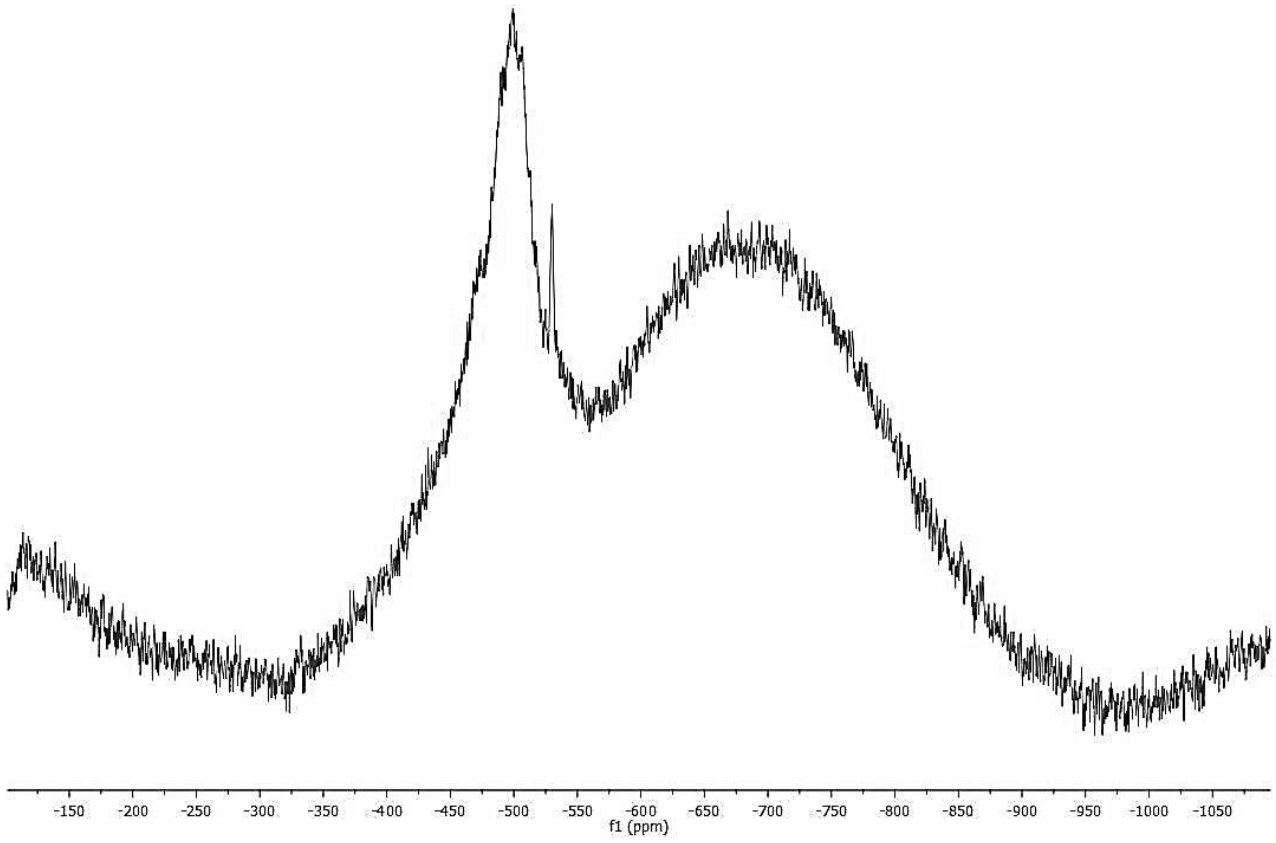


Figure S2. ^{51}V NMR spectrum of **16** (3 mg) recorded after 72 h in solution (DMSO-d6, *ca.* 400 µL).

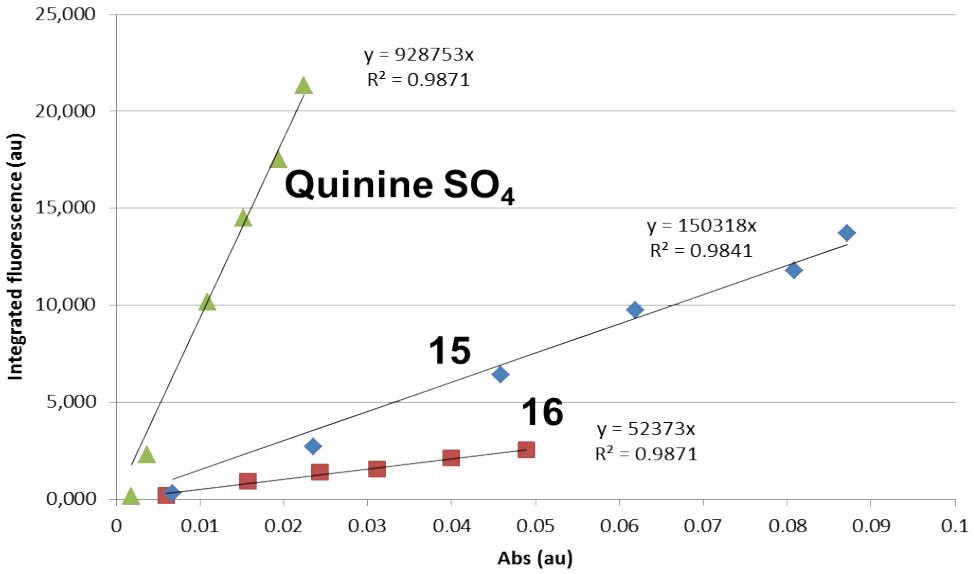
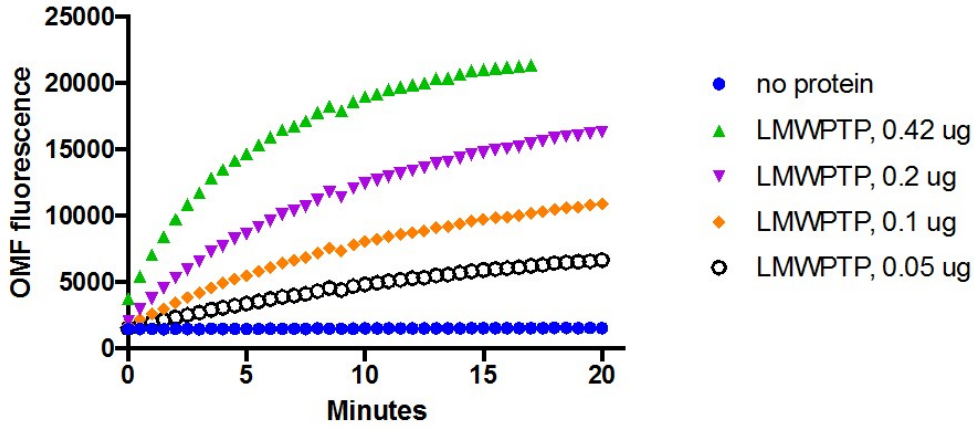


Figure S3. Linear plots for the calculation of fluorescence quantum yields for **15** and **16**. The gradient of each plot (IF vs A) is proportional to the quantum yield of the sample. For each test sample, the Φ_F value is obtained relevant to the standard (quinine sulphate) and represents the quantum yield value calculated.



	no protein	LMW-PTP, 0.05 µg	LMW-PTP, 0.1 µg	LMW-PTP, 0.21 µg	LMW-PTP, 0.42 µg
Slope Values	4.45 ± 0.20	2841 ± 110.5	1424 ± 41.41	669.8 ± 14.95	287.8 ± 5.95

Figure S4. LMW-PTP activity (as an e.g. for all phosphatases) in the presence of OMFP as a substrate. Four slope lines represent measurements of OMFP hydrolysis in the presence of LMW-PTP-GST (0.05 – 0.42 µg). Horizontal line corresponds to the background measurements in the absence of LMW-PTP. High values of the slopes indicate high LMW-PTP phosphatase activity.

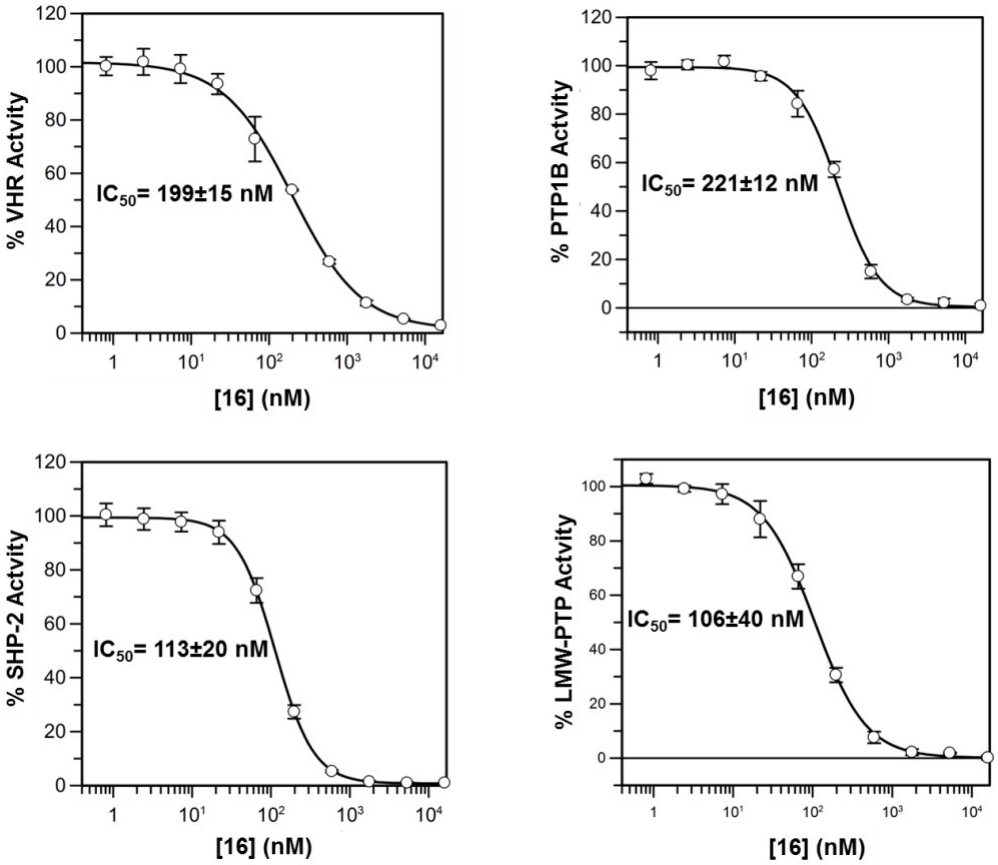


Figure S5. IC_{50} curves (values nM ± standard deviation of triplicate repeats) of dimedone-based $VO(pic)_2$ complex **16** for PTP1B (= protein-tyrosine phosphatase 1B), SHP-2 (= Src homology region 2 domain-containing phosphatase-2), LMW-PTP (= low molecular weight protein tyrosine phosphatase), and VHR (= dual specificity protein phosphatase 3).

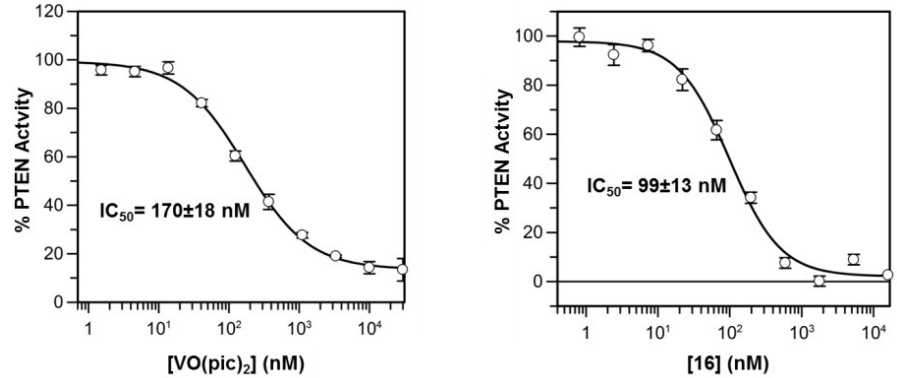


Figure S6. IC_{50} curves (values nM ± standard deviation of triplicate repeats) of $VO(pic)_2$ (reference compound, Figure 1 - main text) and new dimedone-based $VO(pic)_2$ complex **16** for PTEN (= phosphatase and tensin homolog).

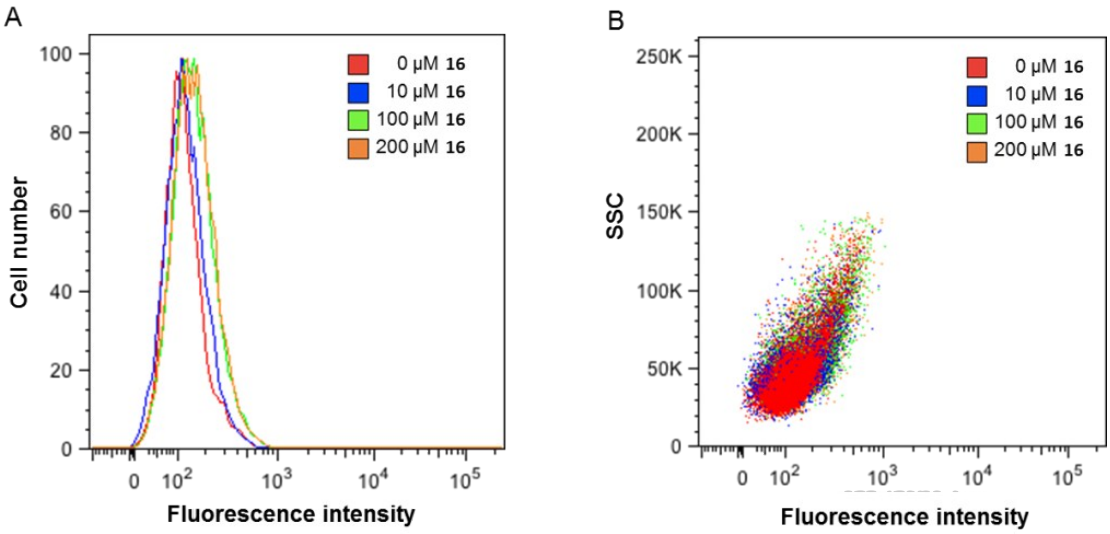


Figure S7. Live cell uptake of **16** (flow cytometry). Live HCT116 were treated with 10-200 μM of **16** for 24 hours. Flow cytometry histogram (**A**) and dotplot (**B**) show no significant increase of fluorescence in HCT116 cells after 24 hours treatment with **16**, indicating that there is no detectable uptake of **16** in live HCT116 cells. Untreated cells are shown in Red. 10000 cells were measured for each analysis. Autofluorescence was measured in the absence of **16** (0 μM).

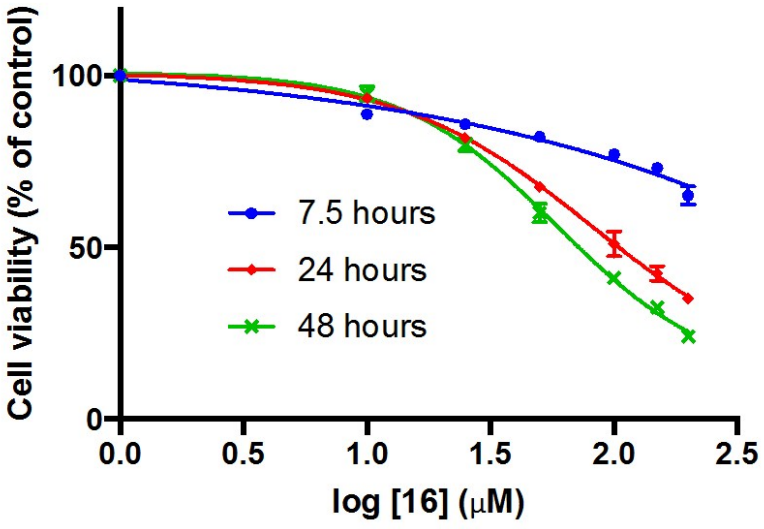


Figure S8. Cytotoxicity of **16**. HCT116 cells were treated with 100 nM PMA and 0 - 200 μM of **16** for 7.5, 24 or 48 hours. Cell viability was measured by MTS assay. Data shown in average of triplicates ± SD%.

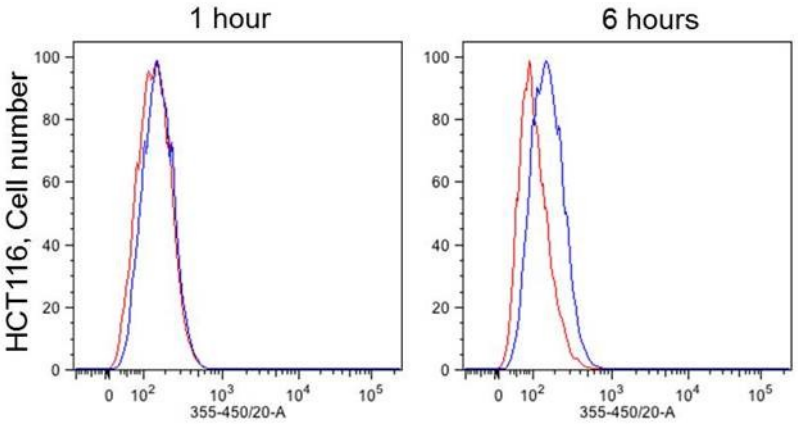


Figure S9. Live cell uptake of **16** + PMA (flow cytometry). Live HCT116 cells were co-treated with 100 nM PMA and 200 µM of **16** for 1 and 6 hours. Flow cytometry histograms show increase of fluorescence in HCT116 cells after 6 hours co-treatment (i.e. PMA + **16**), indicating that PMA treatment facilitates the uptake of **16** in HCT116 cells. Untreated cells are shown in Red. 10000 cells were measured for each analysis.

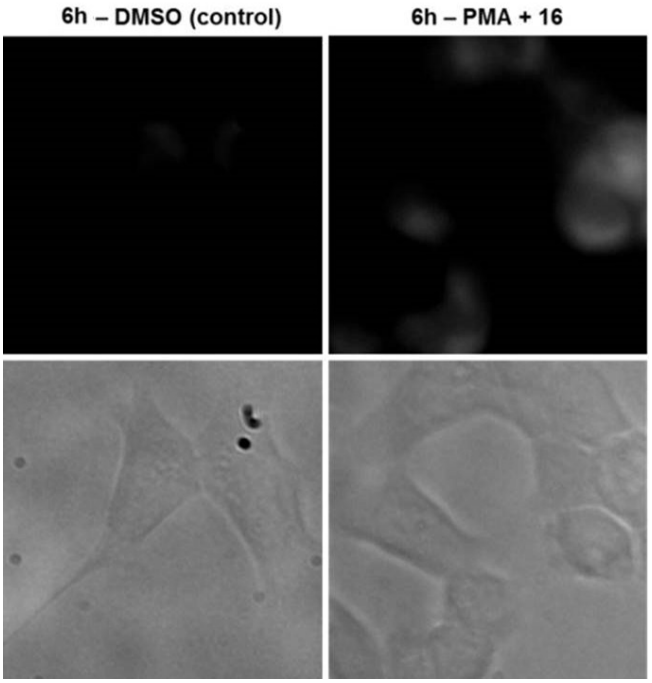


Figure S10. Live cell uptake of **16** + PMA (microscopy). Live HCT116 cells were co-treated with 100 nM PMA and 200 µM of **16** for 6, showing increase of fluorescence and indicating that PMA treatment facilitates the uptake of **16**. Untreated cells are shown at 6 hours (i.e. no incubation with PMA + **16**). Quantification of fluorescence signal was performed with FIJI.

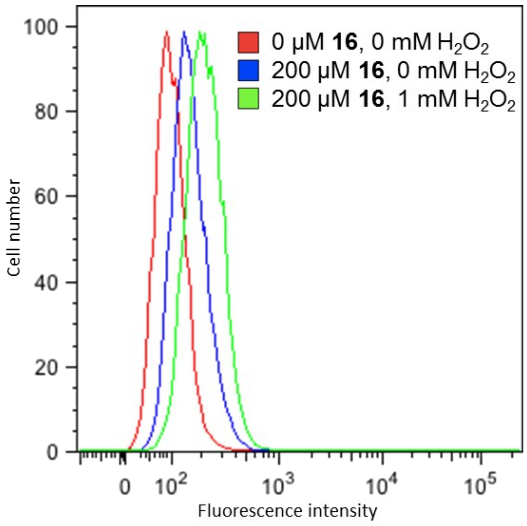


Figure S11. Flow cytometry histogram of HCT116 cells incubated with **16** under oxidative conditions. Live cells were co-treated with 100 nM PMA and **16** (200 μM) for 6 hours following by addition of 0 or 1 mM of H_2O_2 for 30 min. Cells treated with 1 mM H_2O_2 (Green) show an increase in fluorescence intensity compared to the control (i.e. no H_2O_2, Blue). Untreated cells (no **16**, no H_2O_2) are shown in Red. 10000 cells were measured for each analysis.

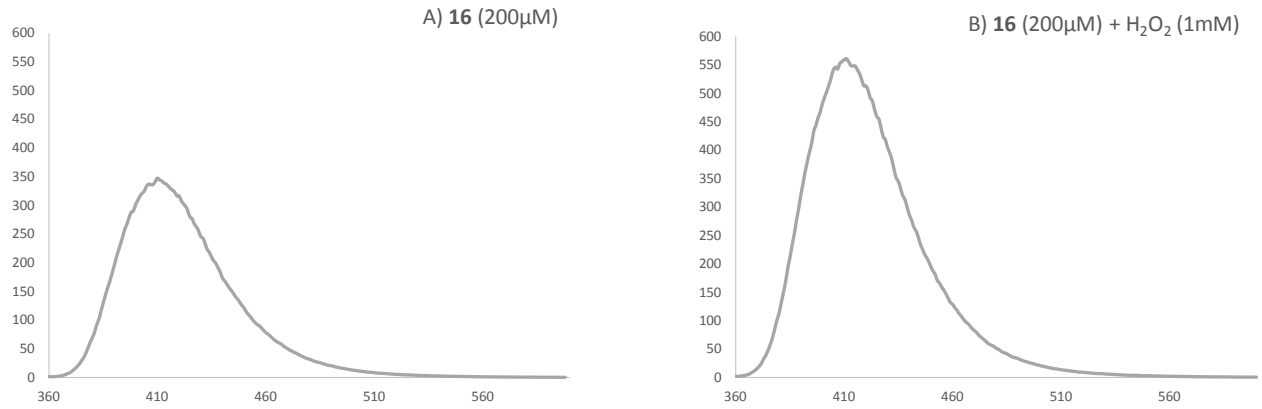


Figure S12. Emission spectra for **16** in the absence/presence of H_2O_2 for 30 min. A) Fluorescence emission of **16** (200 μM) in PBS. B) Fluorescence emission of **16** (200 μM) + 30% H_2O_2 (1mM) in PBS after 30 minutes.