Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Synthesis and optical properties of macrocyclic lanthanide (III) chelates as new reagents for luminescent biolabeling

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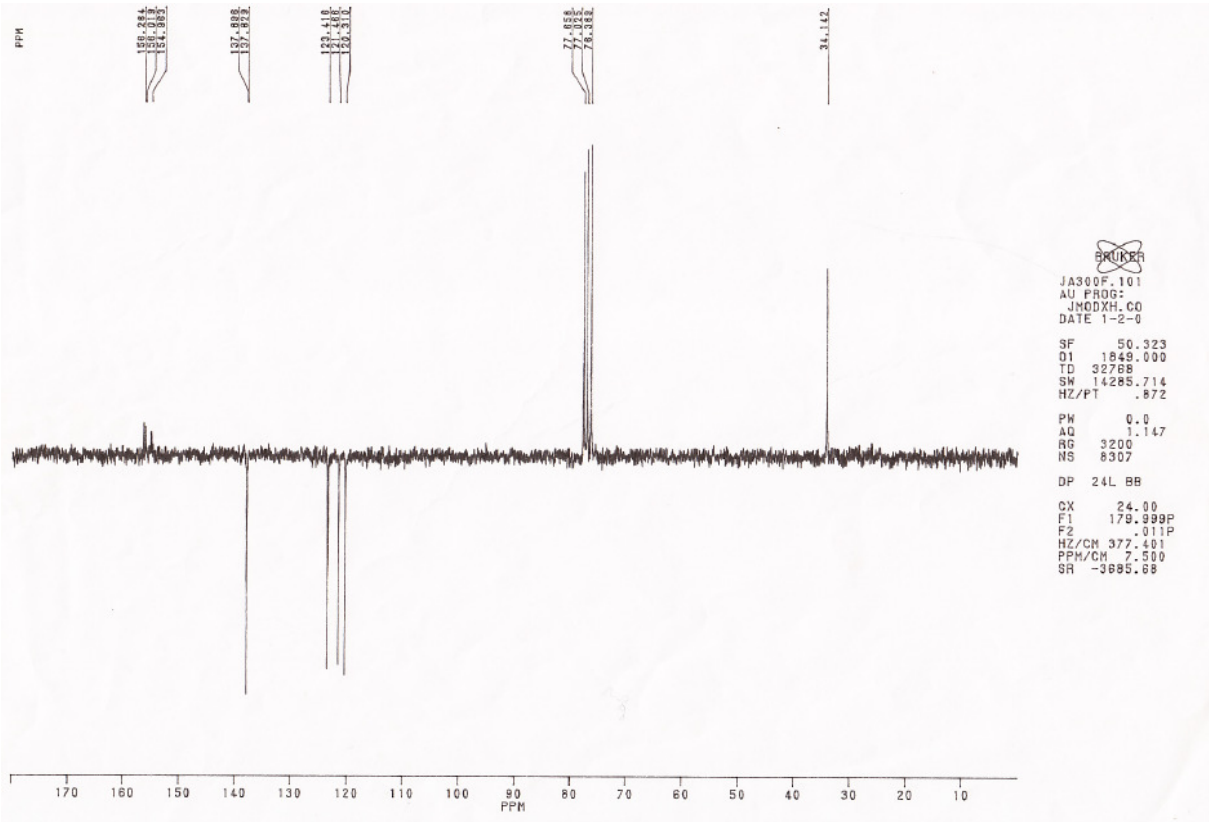
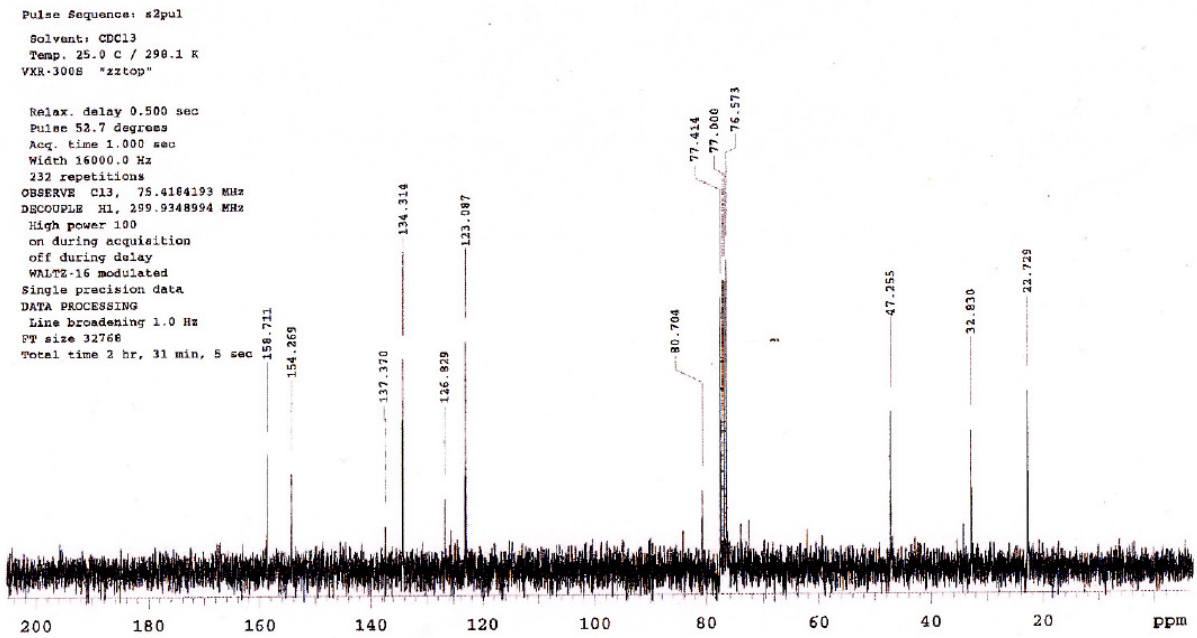
Supporting Information

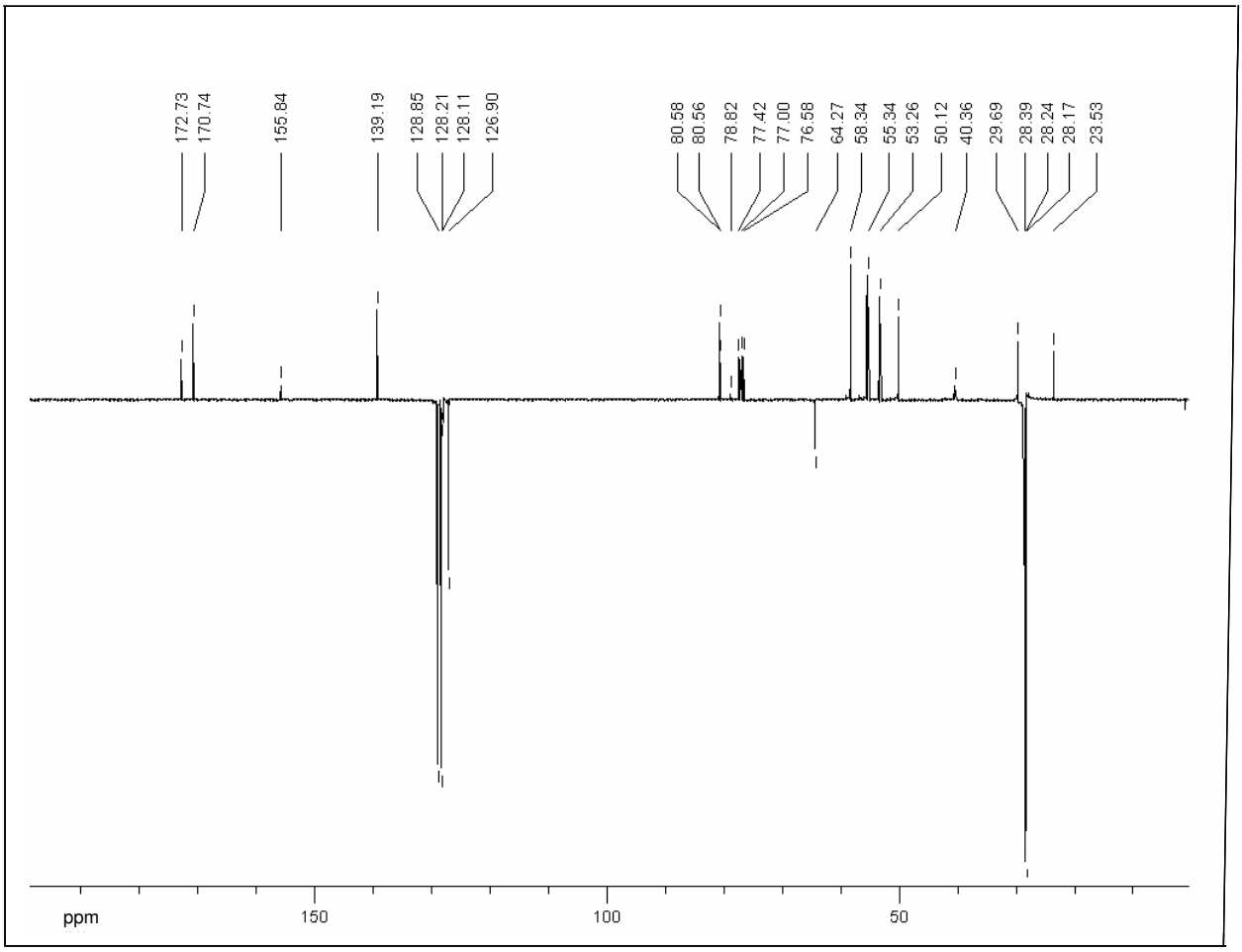
^{13}C NMR spectrum of 6,6''-Bis(bromomethyl)-2,2':6',2''-terpyridine (**12**) recorded in $CDCl_3$ (50 MHz).



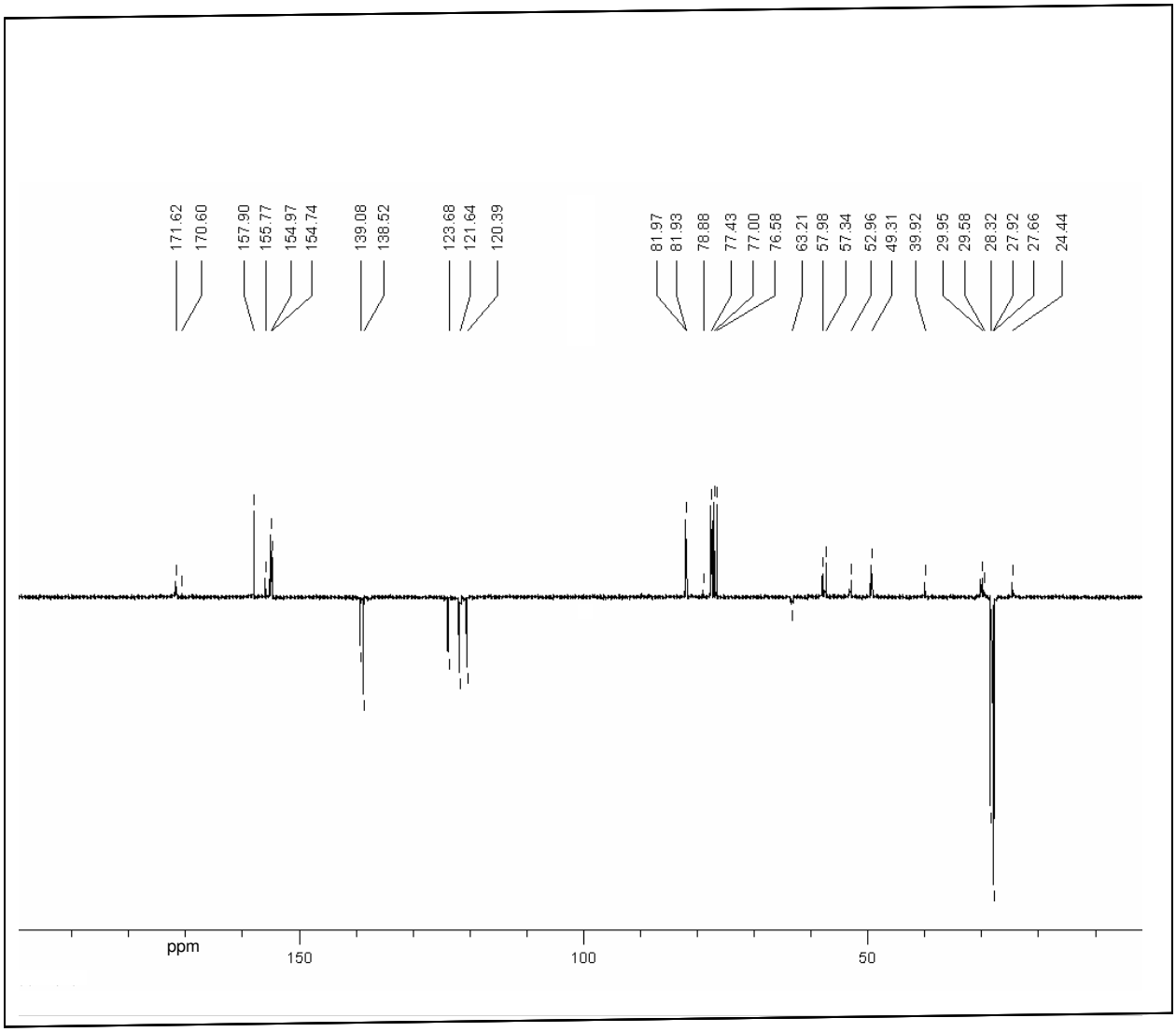
^{13}C NMR spectrum of 3,6-Bis(chloromethyl)-10,11,12,13-tetrahydrodipyrido-[3,2-a:2',3'-c]-phenazine (**13**) recorded in $CDCl_3$ (100 MHz).



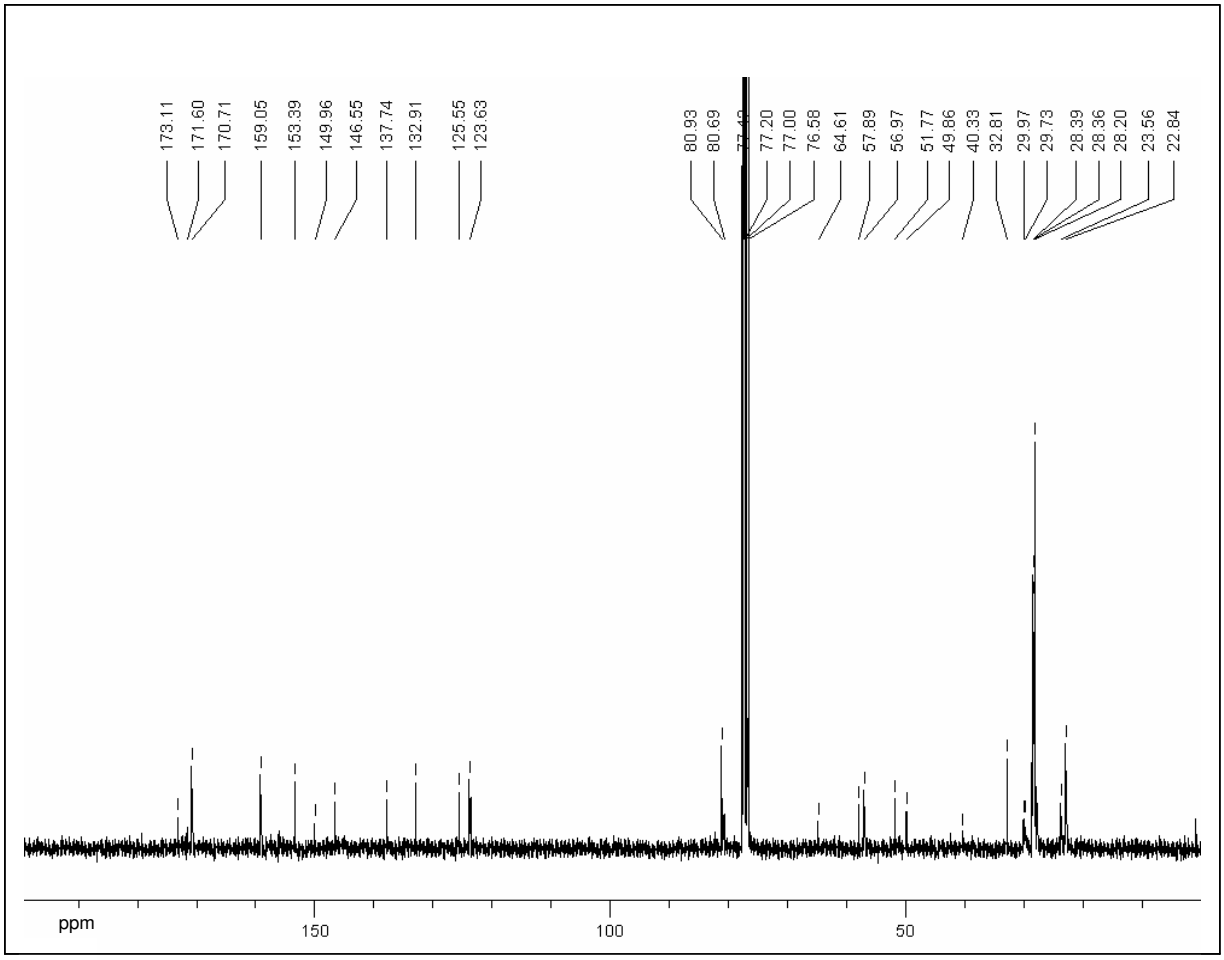
^{13}C NMR spectrum of *N*,*N*"-Dibenzyl protected triamine (**20**) recorded in $CDCl_3$ (75 MHz).



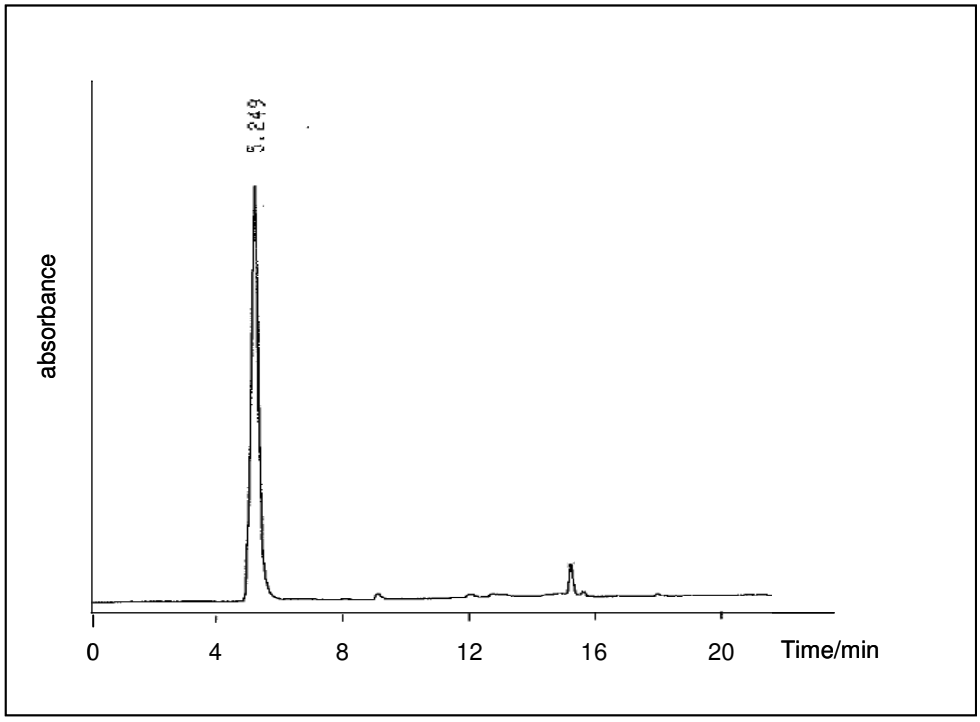
^{13}C NMR spectrum of fully-protected terpyridine (tpy) ligand (**14**) recorded in $CDCl_3$ (75 MHz).



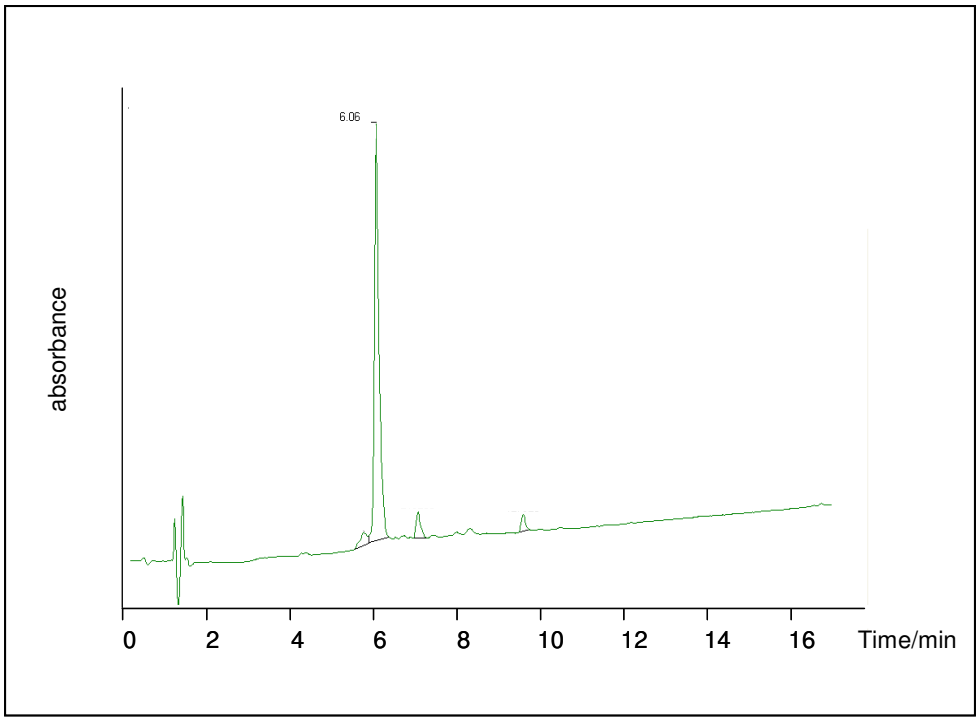
^{13}C NMR spectrum of fully-protected dipyrido-6,7,8,9-tetrahydrophenazine (dpqc) ligand (**16**) recorded in $CDCl_3$ (75 MHz).



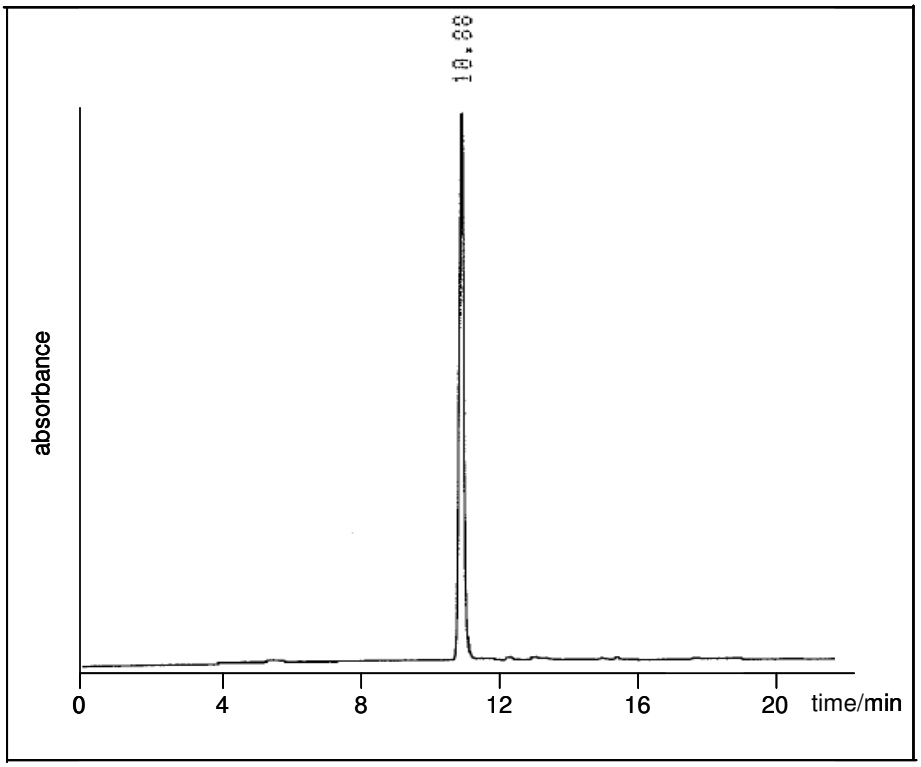
RP-HPLC elution profile (system A) of Terpyridine ligand **15**

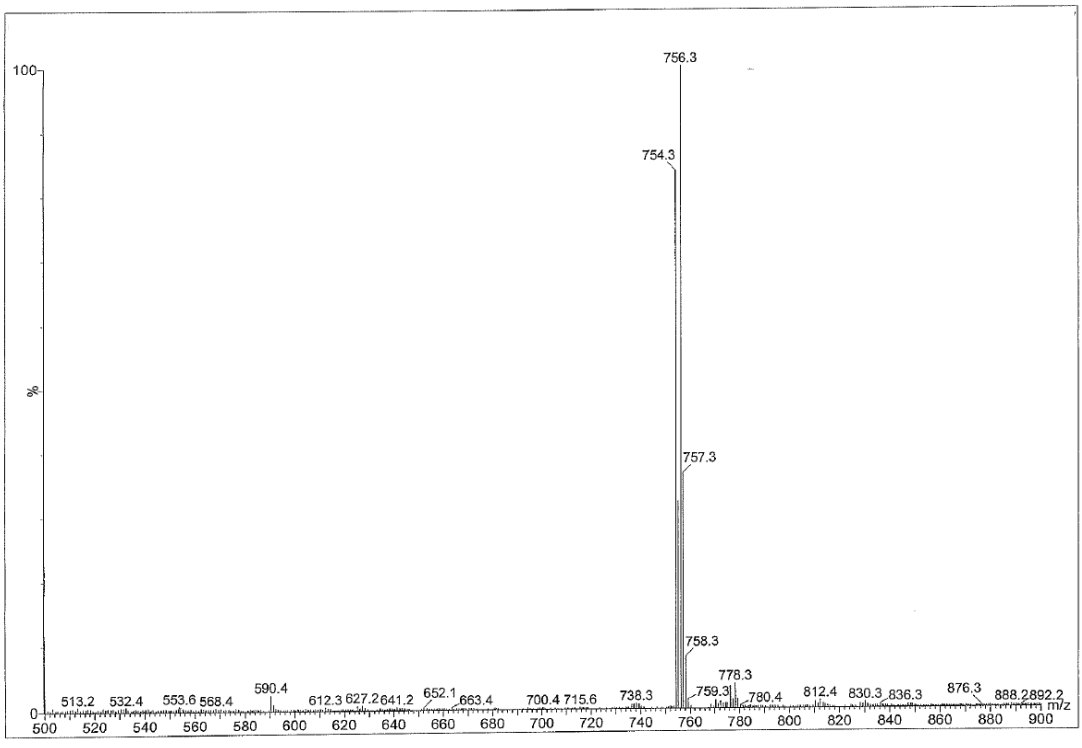


RP-HPLC elution profile (system B) of dipyrido-6,7,8,9-tetrahydrophenazine (dpqc) ligand **17**

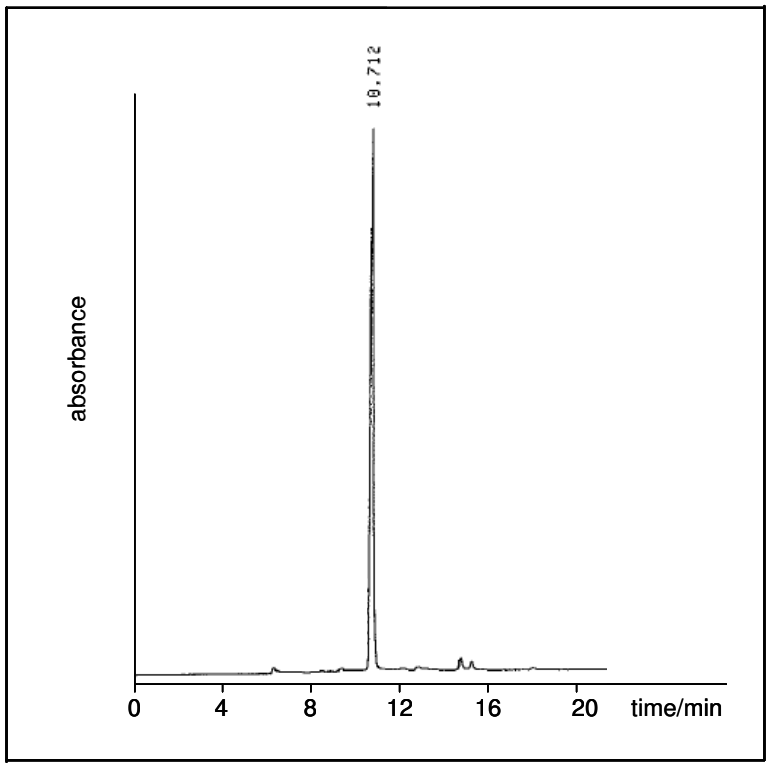


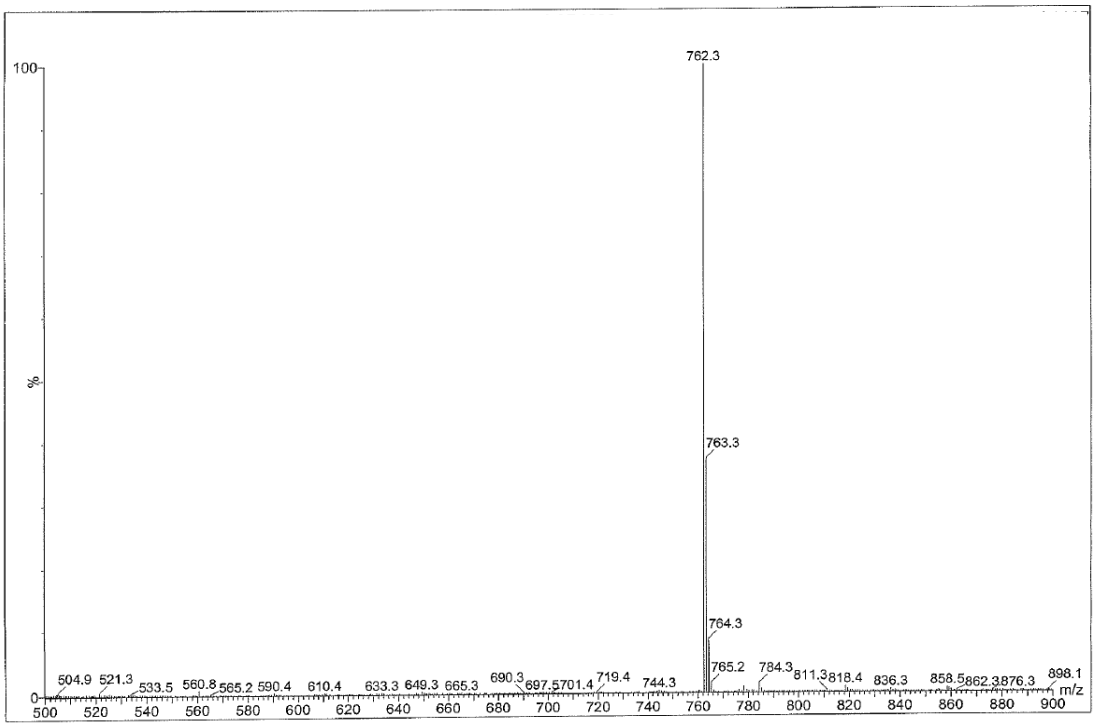
RP-HPLC elution profile (System A) and ESI-MS spectrum of europium complex **5**



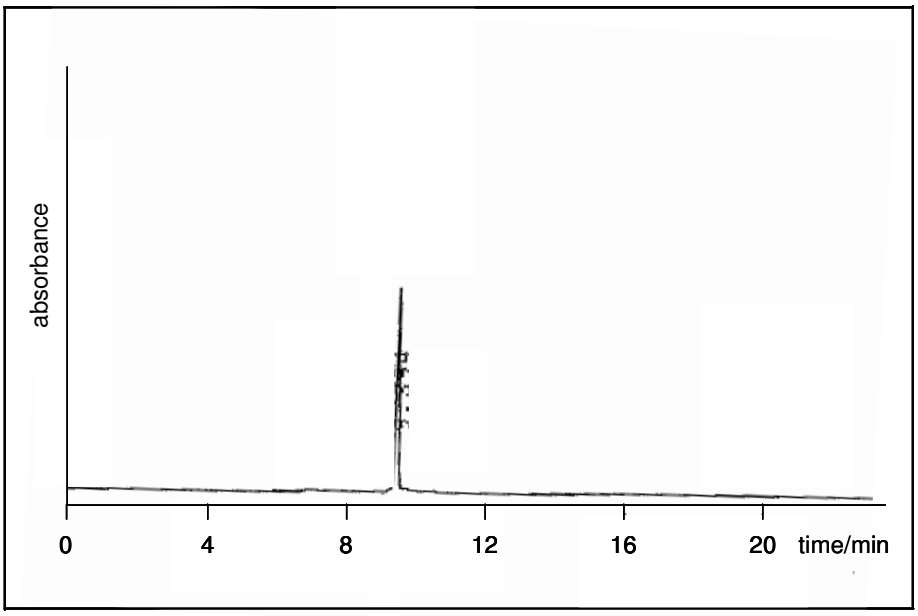


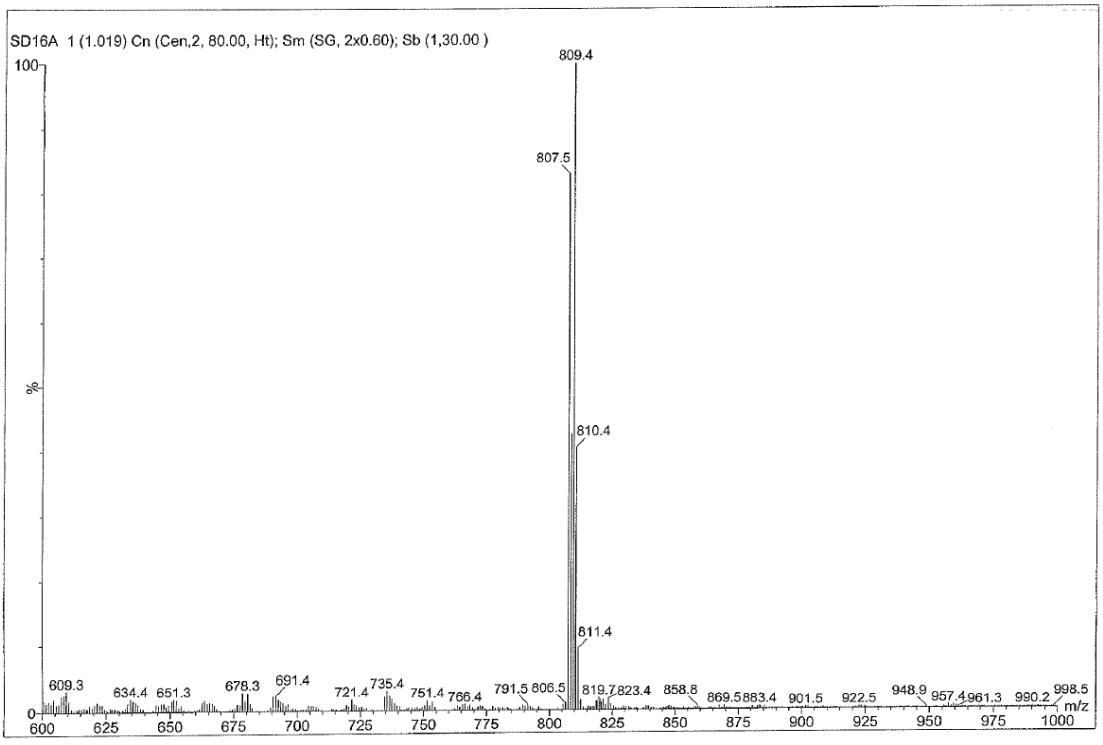
RP-HPLC elution profile (System A) and ESI-MS spectrum of terbium complex **6**



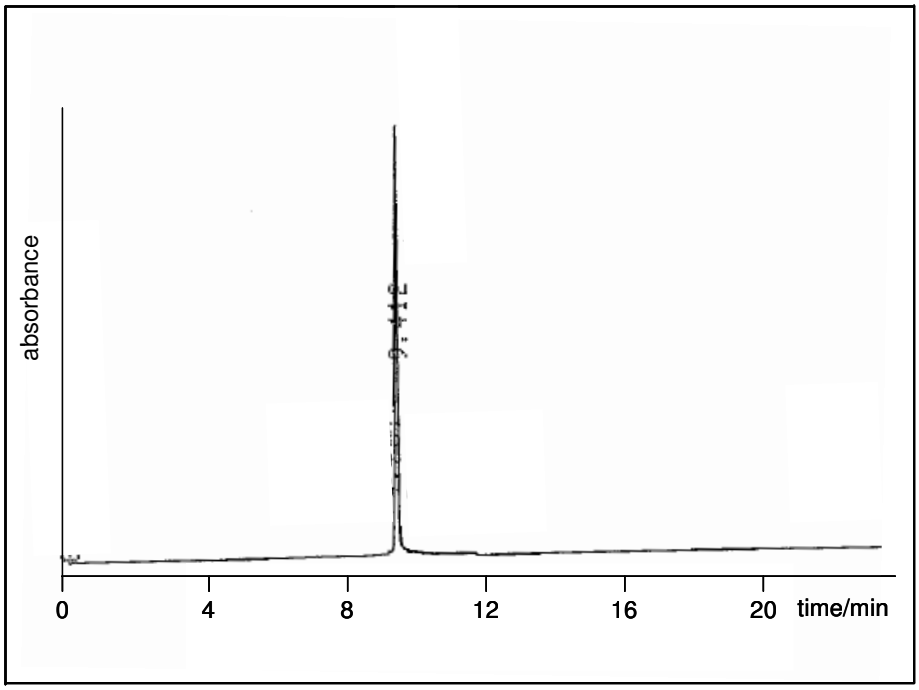


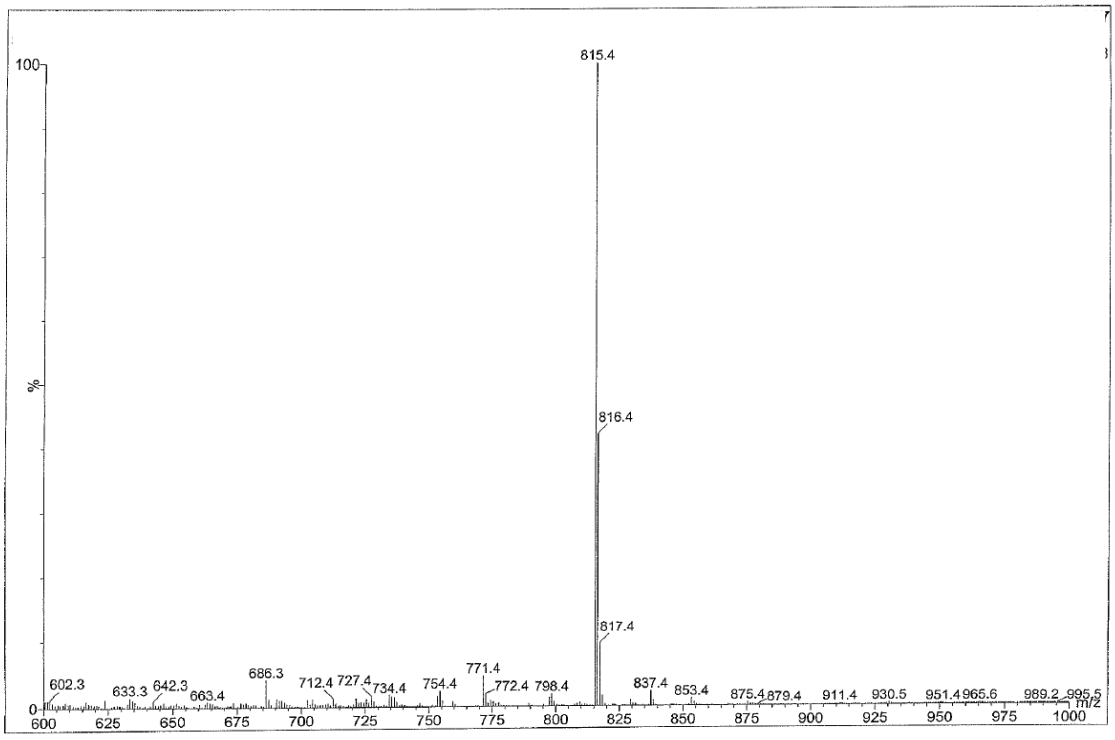
RP-HPLC elution profile (System C) and ESI-MS spectrum of europium complex **7**



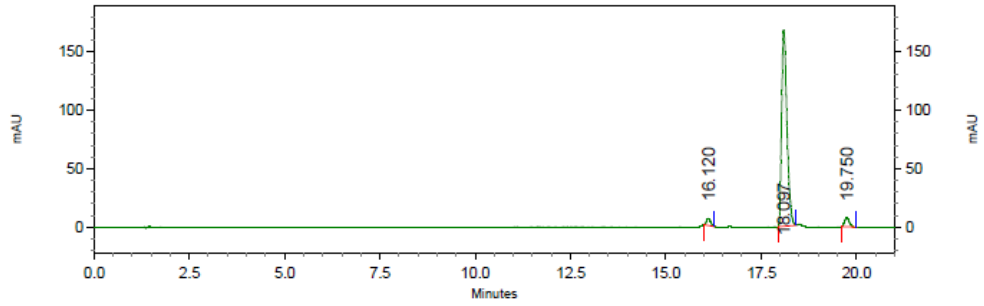


RP-HPLC elution profile (System C) ESI-MS spectrum of terbium complex **8**





RP-HPLC elution profile (System D) of Eu(III) chelate **9**



Retention Time	Area	Area %	Height	Height %
16.120	44443	2.56	6034	3.32
18.097	1624590	93.67	168260	92.50
19.750	65306	3.77	7610	4.18
Totals	1734339	100.00	181904	100.00

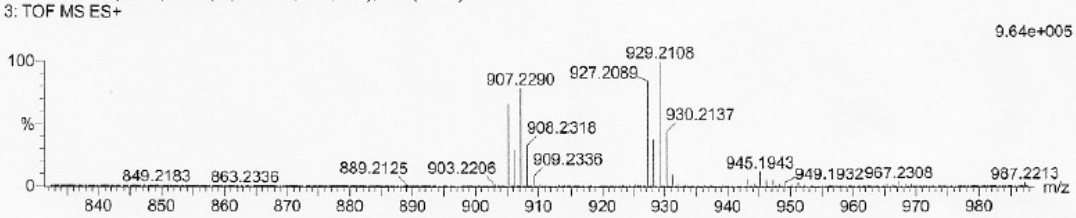
High-resolution mass spectrum of Eu(III) chelate **9**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
631 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-10 O: 0-10 151Eu: 0-1

Coll Energy = 6 Cone Voltage = 50 XEVO-G2QTOF#YCA210
TPYMAIEu 33 (0.522) AM2 (Ar,14000.0,0.00,0.00); Cm (31:35)
3: TOF MS ES+



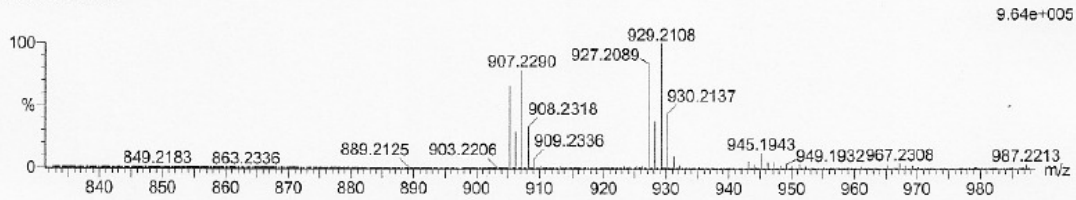
Minimum:				-1.5				
Maximum:		3.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
905.2270	905.2273	-0.3	-0.3	22.5	182.9	0.888	41.17	C38 H42 N8 O9 151Eu
	905.2255	1.5	1.7	35.5	183.0	0.944	38.92	C50 H38 N6 O2 151Eu
	905.2287	-1.7	-1.9	22.0	183.6	1.614	19.91	C40 H44 N5 O10 151Eu

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

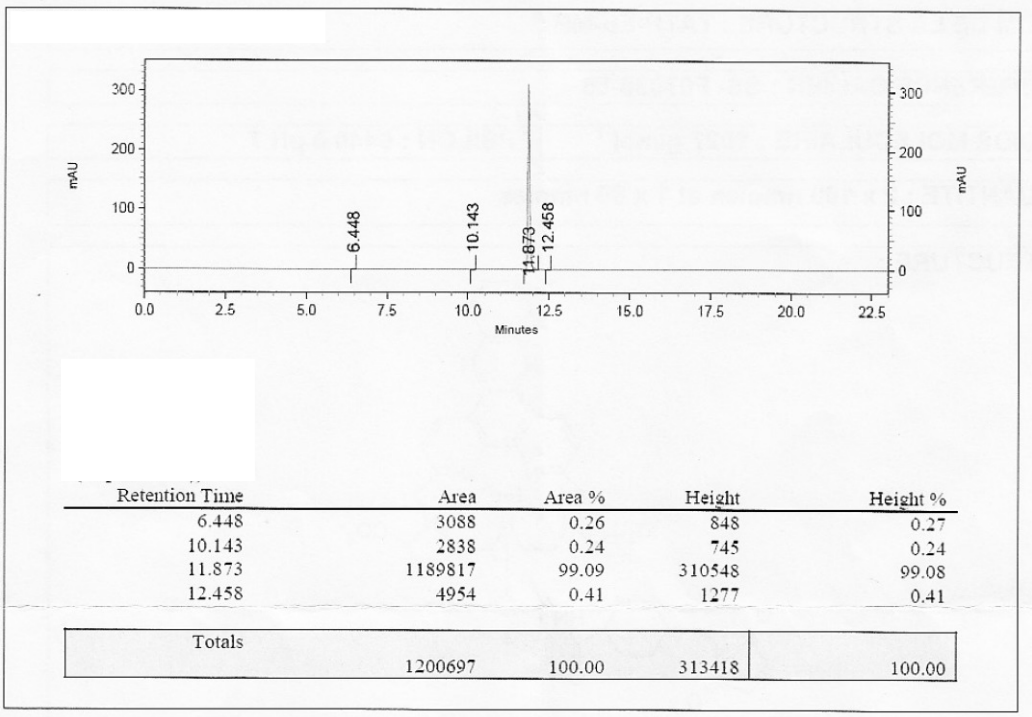
Monoisotopic Mass, Even Electron Ions
633 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-10 O: 0-10 151Eu: 0-1 Na: 1-1

Coll Energy = 6 Cone Voltage = 50 XEVO-G2QTOF#YCA210
TPYMAIEu 33 (0.522) AM2 (Ar,14000.0,0.00,0.00); Cm (31:35)
3: TOF MS ES+



Minimum:				-1.5				
Maximum:		3.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
927.2089	927.2093	-0.4	-0.4	22.5	161.3	0.688	50.24	C38 H41 N8 O9 151Eu Na
	927.2074	1.5	1.6	35.5	161.3	0.698	49.76	C50 H37 N6 O2 151Eu Na

RP-HPLC elution profile (System E) of Eu(III) chelate **10**



Retention Time	Area	Area %	Height	Height %
6.448	3088	0.26	848	0.27
10.143	2838	0.24	745	0.24
11.873	1189817	99.09	310548	99.08
12.458	4954	0.41	1277	0.41
Totals	1200697	100.00	313418	100.00

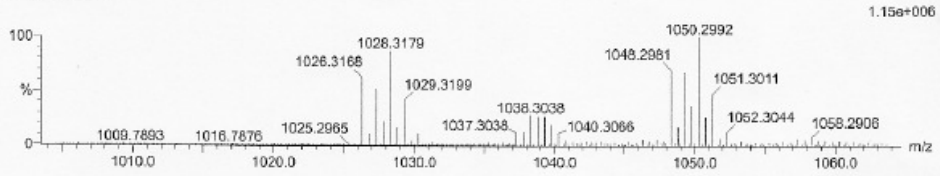
High-resolution mass spectrum of Eu(III) chelate **10**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 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
278 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-10 O: 0-10 151Eu: 0-1

Coll Energy = 6 Cone Voltage = 50 XEVO-G2QTOF#YCA210
TATPMAIEu 39 (0.612) AM2 (Ar,14000.0,0.00,0.00); Cm (35:43)
3: TOF MS ES+



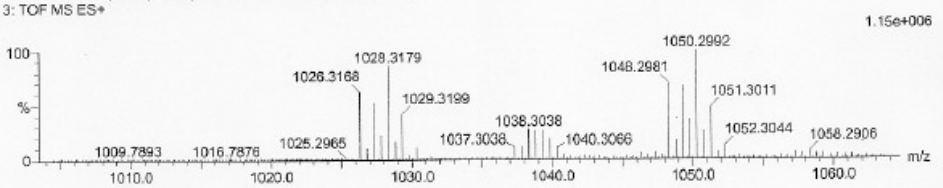
Minimum:				-1.5				
Maximum:		3.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1026.3168	1026.3165	0.3	0.3	25.5	145.3	n/a	n/a	C46 H53 N9 O9 151Eu

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

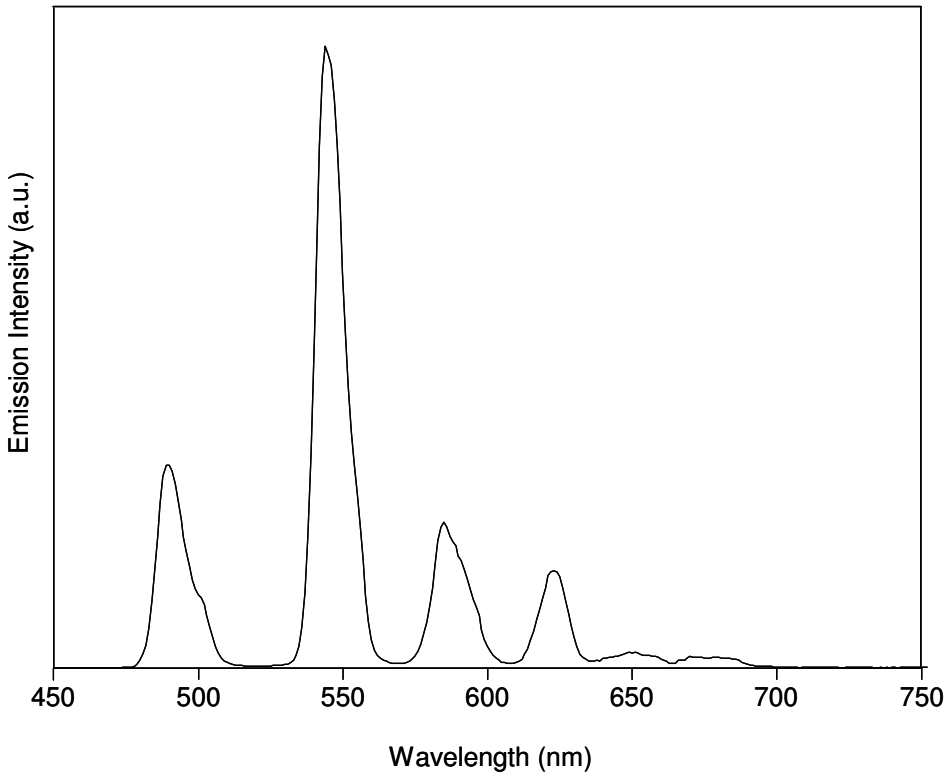
Monoisotopic Mass, Even Electron Ions
283 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-10 O: 0-10 151Eu: 0-1 Na: 1-1

Coll Energy = 6 Cone Voltage = 50 XEVO-G2QTOF#YCA210
TATPMAIEu 39 (0.612) AM2 (Ar,14000.0,0.00,0.00); Cm (35:43)
3: TOF MS ES+

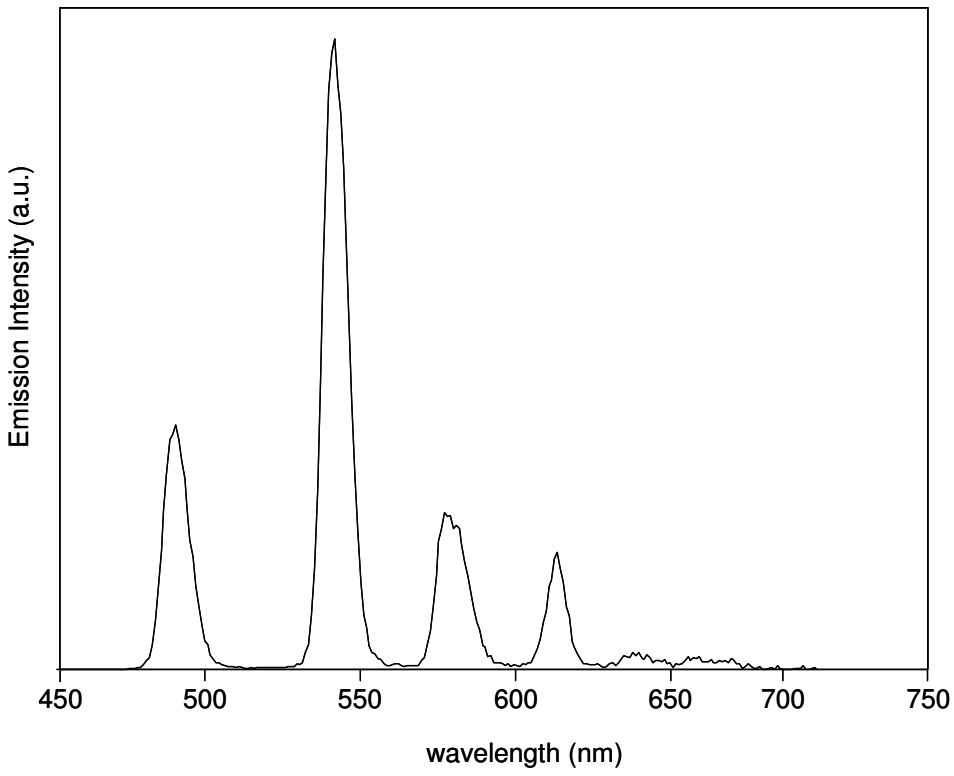


Minimum:				-1.5				
Maximum:		3.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1048.2981	1048.2984	-0.3	-0.3	25.5	210.3	n/a	n/a	C46 H52 N9 O9 151Eu Na

Corrected emission spectrum of terbium complex **6** (1×10^{-6} M in Tris buffer 50 mM, pH 7.4) at 298 K.



Corrected emission spectrum of terbium complex **8** (1×10^{-6} M in Tris buffer 50 mM, pH 7.4) at 298K.



Quantum yields and lifetimes of Eu(III) complexes **5** and **7** in various media at 298K

Eu(III) complex 5

	Tris buffer (0.05 M, pH 7.3)	Tris buffer + KF (0.4 M)	HEPES buffer (0.05M, pH 7.3)	Phosphate buffer (0.05 M, pH 7.3)	Borate buffer (0.05M, pH 8.6)
Φ (%)	13	14	13	14	13
τ (ms)	1.10	1.10	1.08	1.11	1.10

Eu(III) complex 7

	Tris buffer (0.05 M, pH 7.3)	Tris buffer + KF (0.4 M)	HEPES buffer (0.05M, pH 7.3)	Phosphate buffer (0.05 M, pH 7.3)	Borate buffer (0.05M, pH 8.6)
Φ (%)	3	4	2.5	3	3
τ (ms)	0.46	0.82	0.45	0.58	0.51

Calculated values of η_{sens} for Eu(III) complexes

The overall luminescence quantum yield of the complex (Φ_{obs}) can be broken into two contributions: $\Phi_{obs} = \eta_{sens} \times \Phi_{Eu}$, where η_{sens} is the efficiency of the sensitization of the Eu^{3+} ion through the ligand and Φ_{Eu} is the intrinsic quantum yield of the Eu^{3+} ion when it is excited in its own levels. The latter can be calculated from the observed luminescence lifetime which is influenced by non-radiative processes ($\tau_{obs} = (k_r + \Sigma k_{nr})^{-1}$) and the radiative lifetime τ_R which is not affected by these processes ($\tau_R = k_r^{-1}$). In the particular case of Eu(III), τ_R can be evaluated from the spectral intensity and by using eq (2)

$$\Phi_{Eu} = \tau_{obs} / \tau_R \quad (1)$$

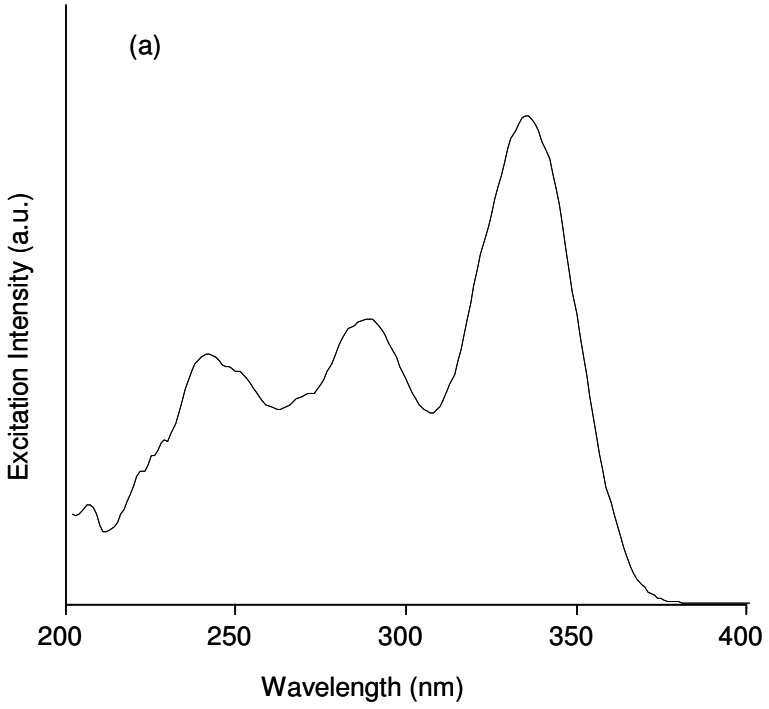
$$1 / \tau_R = k_r = 14.65 \times n^3 \times (I_{\Sigma Fj} / I_{F1}) \quad (2)$$

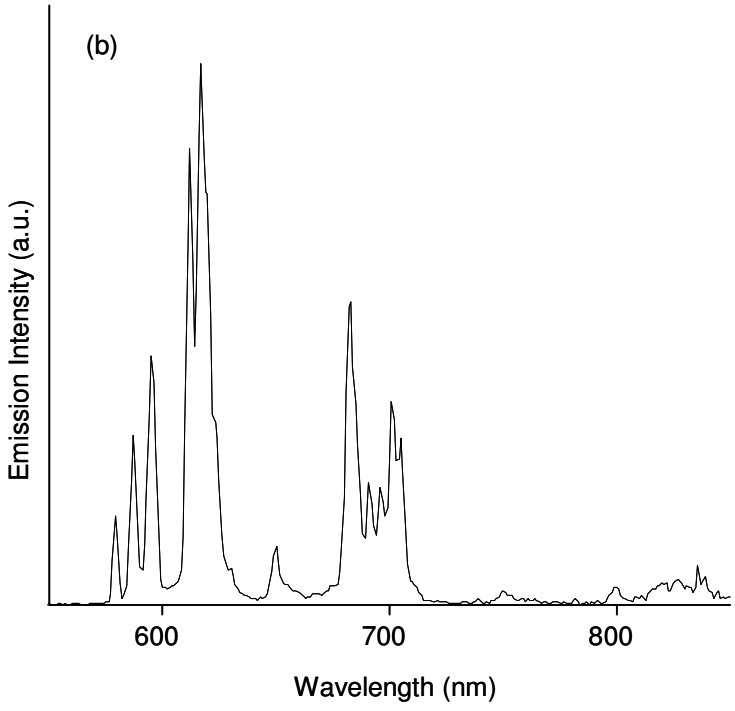
n is the refractive index of the solution, $I_{\Sigma Fj}$ is the total area of the corrected Eu(III) emission spectrum from the $^5D_0 \rightarrow {}^7F_j$ (J = 0-4) and I_{F1} the area of the magnetic dipole transition $^5D_0 \rightarrow {}^7F_1$.

Calculated values of τ_R, k_r, Σk_{nr}, Φ_{Eu} and η_{sens} for Eu(III) complexes complexes using experimentally determined quantities τ_{obs}, Φ and $[I_{F1}/I_{\Sigma F0\text{-}4}]$ in Tris-buffer (pH 7.4) at 298K.

Chelate	$[I_{F1}/I_{\Sigma F0\text{-}4}]$	τ_R [ms]	k_r [s^{-1}]	Σk_{nr} [s^{-1}]	Φ_{Eu} [$\times 10^2$)]	η_{sens} [$\times 10^2$]
[**4**-3H].Eu	0.15	4.35	230	679	25	68
5	0.14	4.07	246	663	27	48
7	0.13	3.77	265	1909	12	25
9	0.14	4.07	246	587	29	52
10	0.13	3.77	265	1553	15	40

(a) Excitation (Iem = 617 nm) and (b) corrected emission (lexc = 334 nm) spectra of **9**-GSS11 conjugate in phosphate buffered saline (100 mM with 0.1% BSA; pH 7) at 298 K. Excitation and emission band passes 20-2.5 nm respectively; delay time 0.1 ms, gate time 0.4 ms.





(a) excitation (lem = 617 nm) and (b) corrected emission (lexc = 350 nm) spectra of **10**-GSS11 conjugate in phosphate buffered saline (100 mM with 0.1% BSA; pH 7) at 298 K. Excitation and emission band passes 20-2.5 nm respectively; delay time 0.1 ms, gate time 0.4 ms.