Electronic Supplementary Information for

New synthesis of phenyl-isothiocyanates *C*-functionalised cyclams. Bioconjugation and ^{64}Cu phenotypic PET imaging studies of multiple myeloma with the te2a derivative

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[b.] *Centre de Recherche en Cancérologie Nantes-Angers (CRCNA), Unité INSERM 892 - CNRS 6299, 8 quai Moncousu, BP 70721 44007 Nantes Cedex, France.*
[c.] *Institut de Cancérologie de l'Ouest, 44800 Saint-Herblain, France.*

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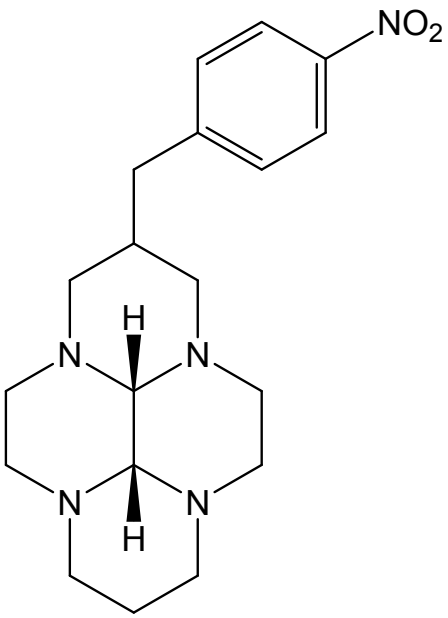
Compound 4:



Chemical Formula: $C_{19}H_{27}N_5O_2$
Exact Mass: 357,2165
Molecular Weight: 357,4500
m/z: 357.2165 (100.0%), 358.2198 (20.5%), 359.2232 (2.0%), 358.2135 (1.8%)

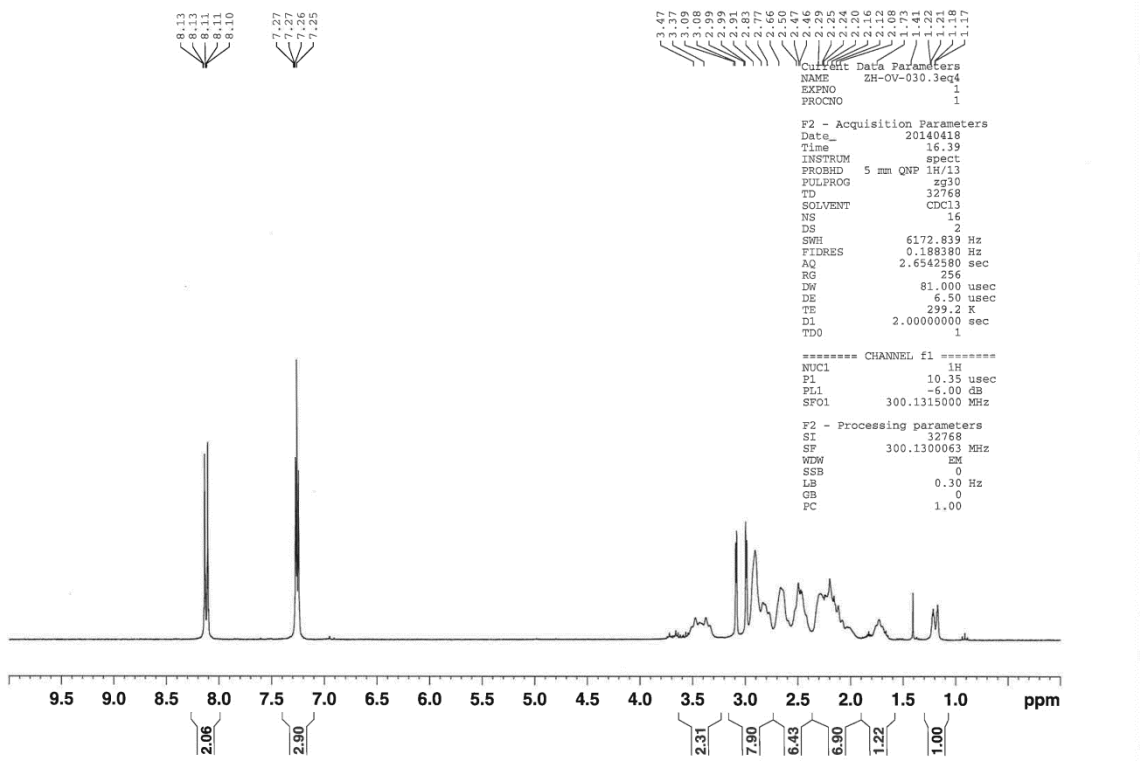


Figure S1. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **4**

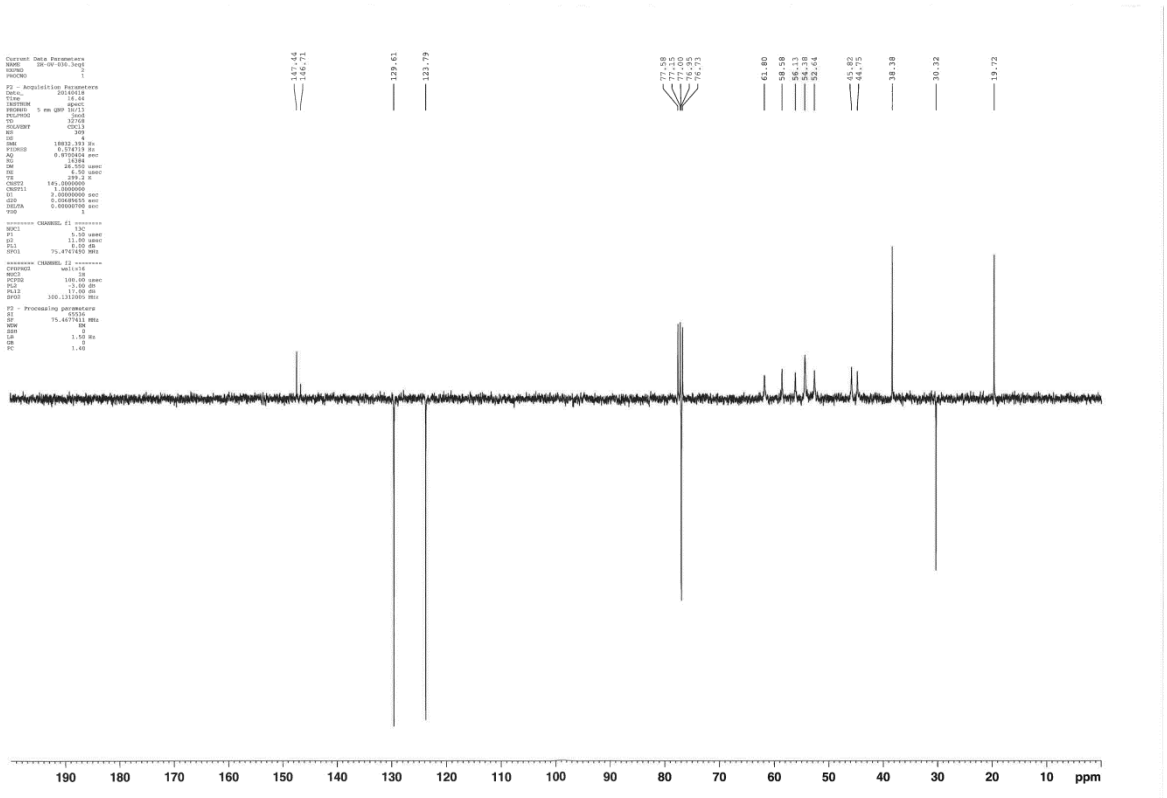


Figure S2. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **4**

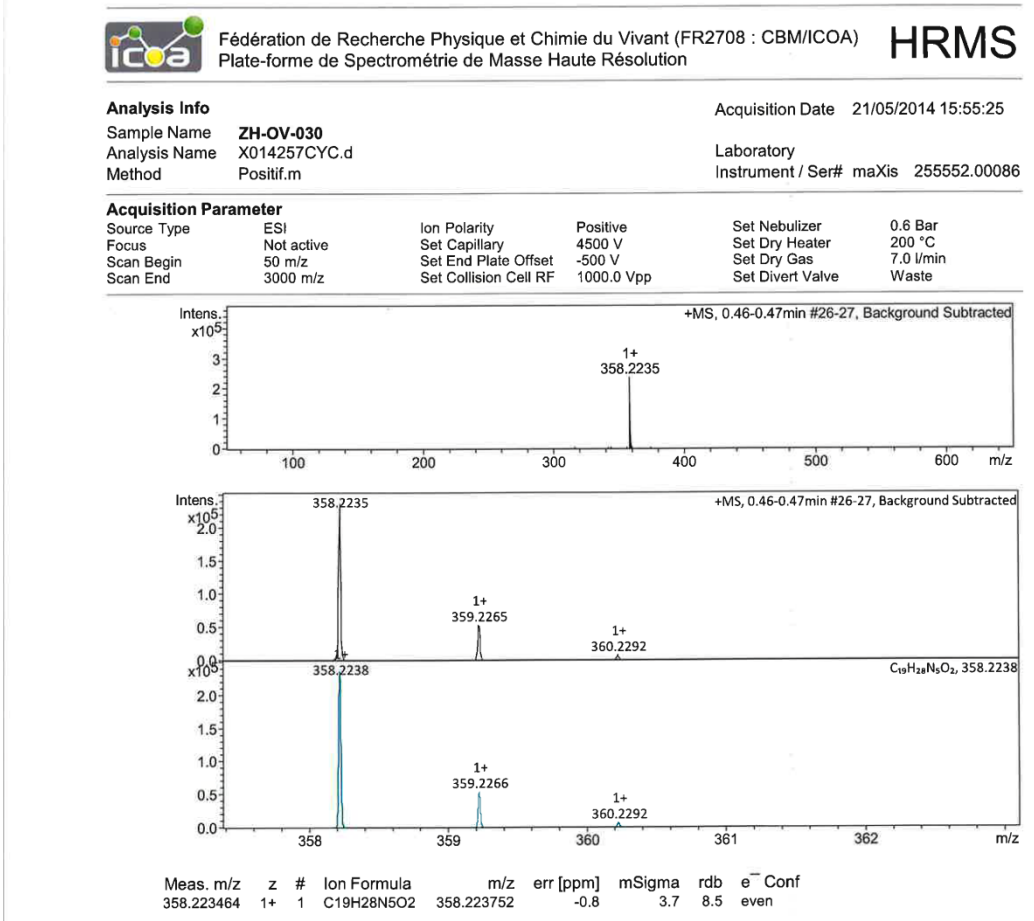
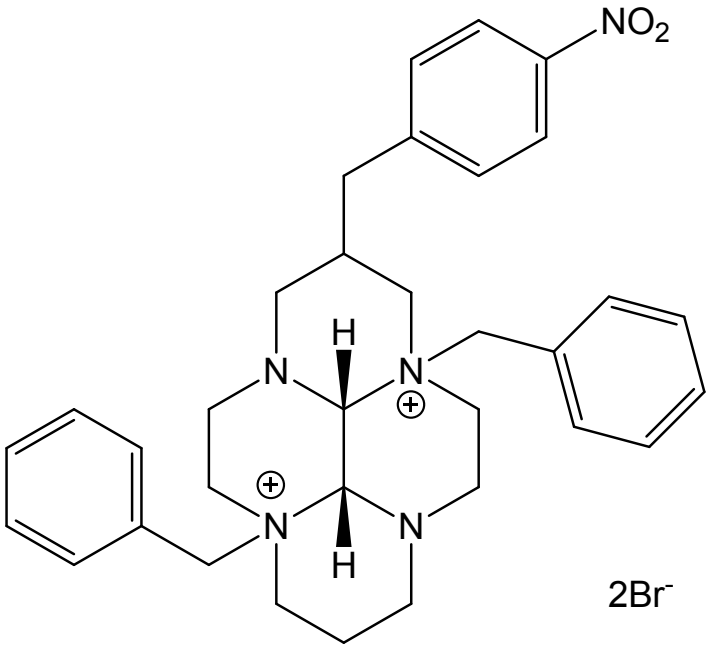


Figure S3. HRMS spectrum (ESI) of compound **4**

Compound 5:



Chemical Formula: $C_{33}H_{41}Br_2N_5O_2$
Exact Mass: 697,1627
Molecular Weight: 699,5189
m/z: 699.1607 (100.0%), 697.1627 (51.4%), 701.1586 (48.6%), 700.1640 (35.7%), 698.1661 (18.3%), 702.1620 (17.4%), 701.1674 (6.2%), 699.1694 (3.2%), 703.1653 (3.0%), 700.1577 (1.8%)

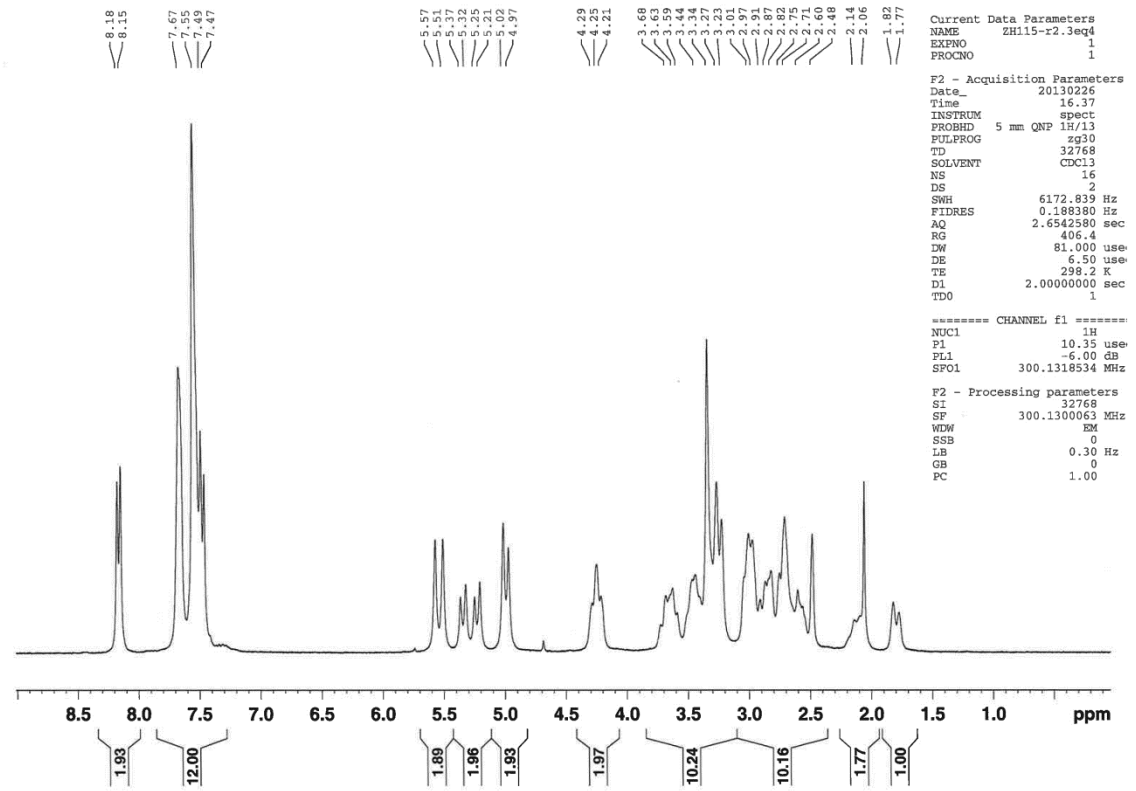


Figure S4. ^{1}H NMR spectrum (300 MHz, ^{6}d-DMSO, 25 °C) of compound **5**

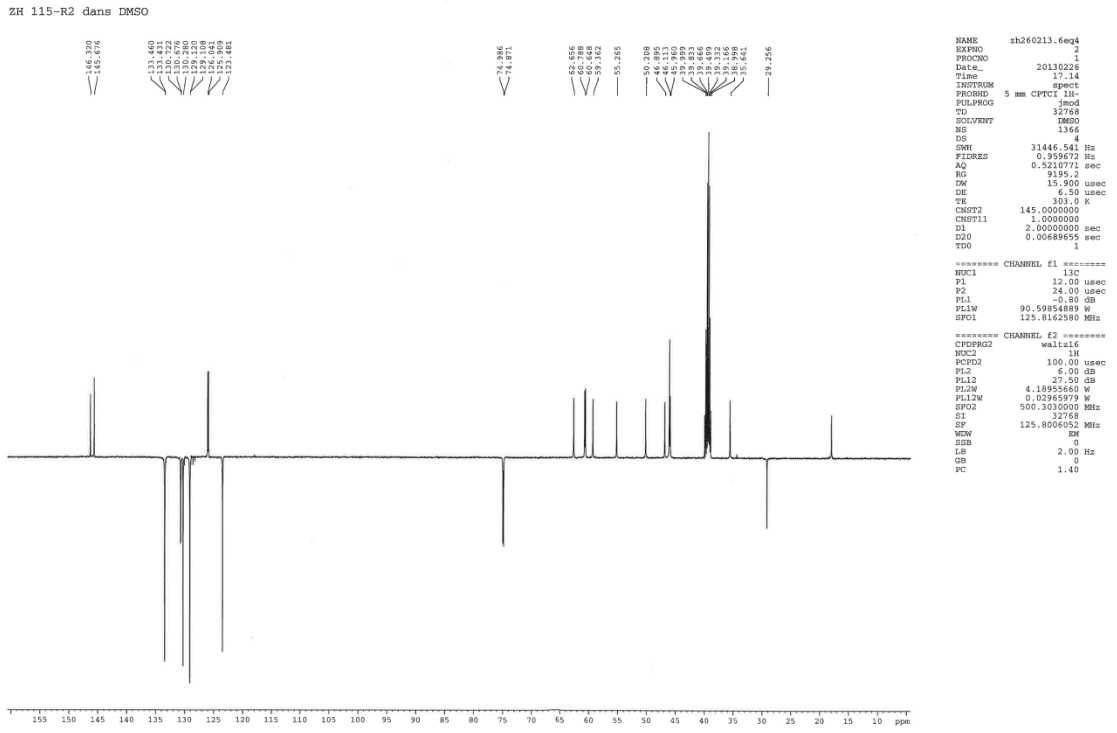


Figure S5. ^{13}C NMR spectrum (300 MHz, ^{6}d-DMSO, 25 °C) of compound **5**

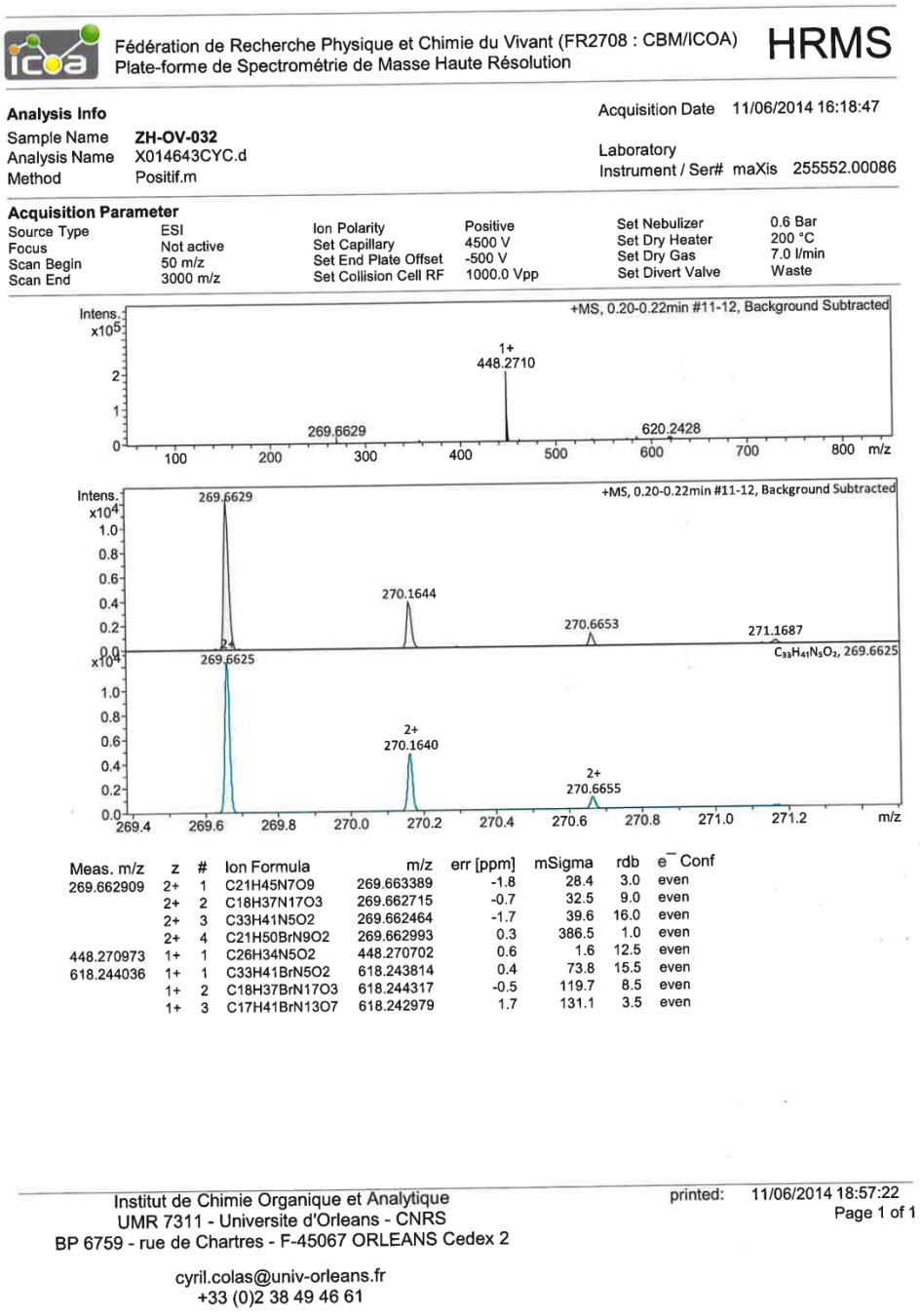
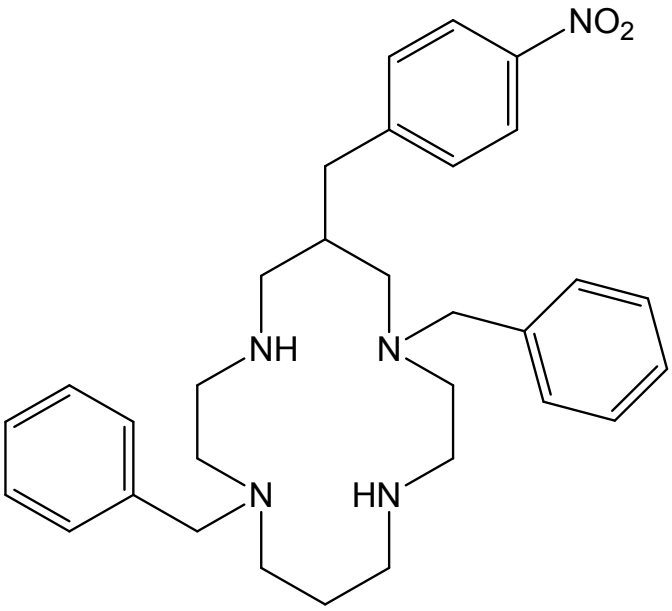


Figure S6. HRMS spectrum (ESI) of compound **5**

Compound 6:



Chemical Formula: $C_{31}H_{41}N_5O_2$
Exact Mass: 515,3260
Molecular Weight: 515,6895
m/z: 515.3260 (100.0%), 516.3294 (33.5%), 517.3327 (5.4%), 516.3231 (1.8%)

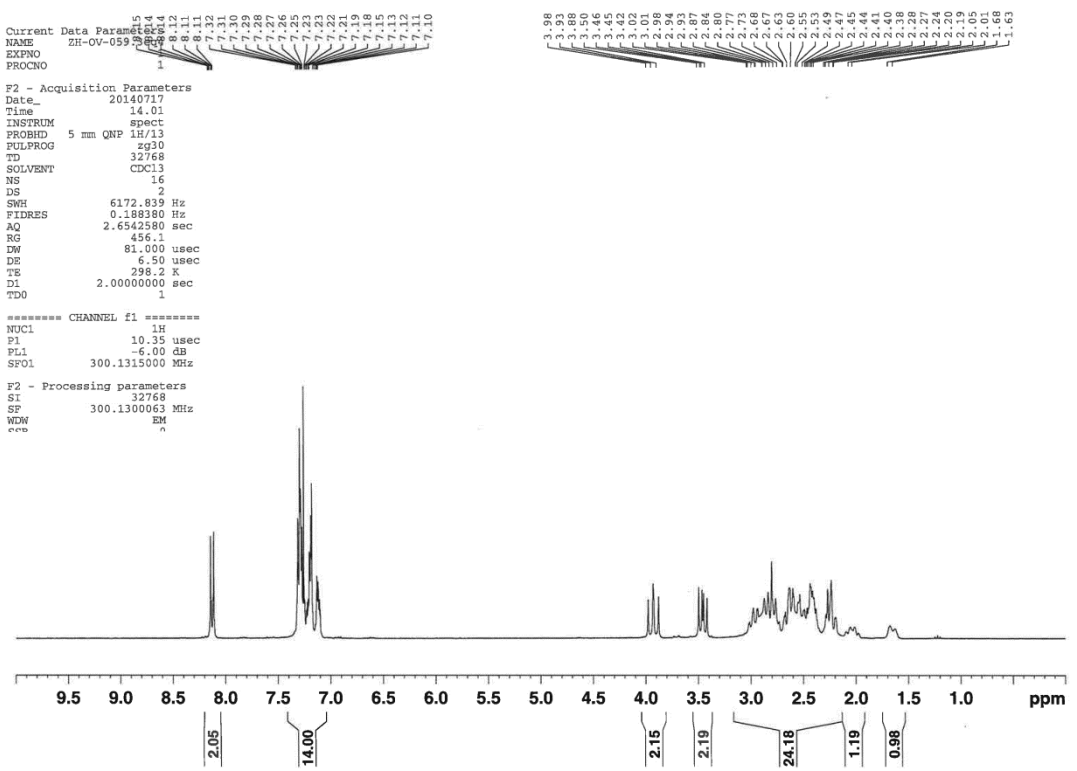


Figure S7. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **6**

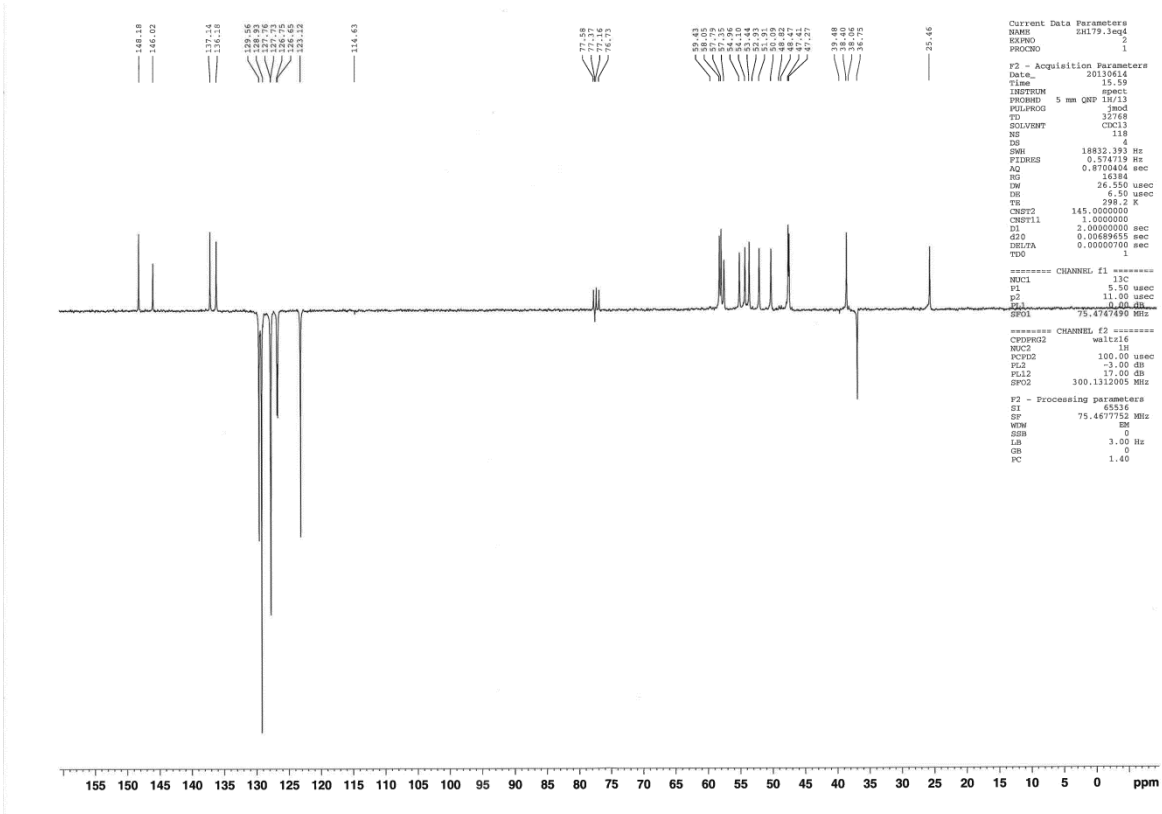


Figure S8. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **6**

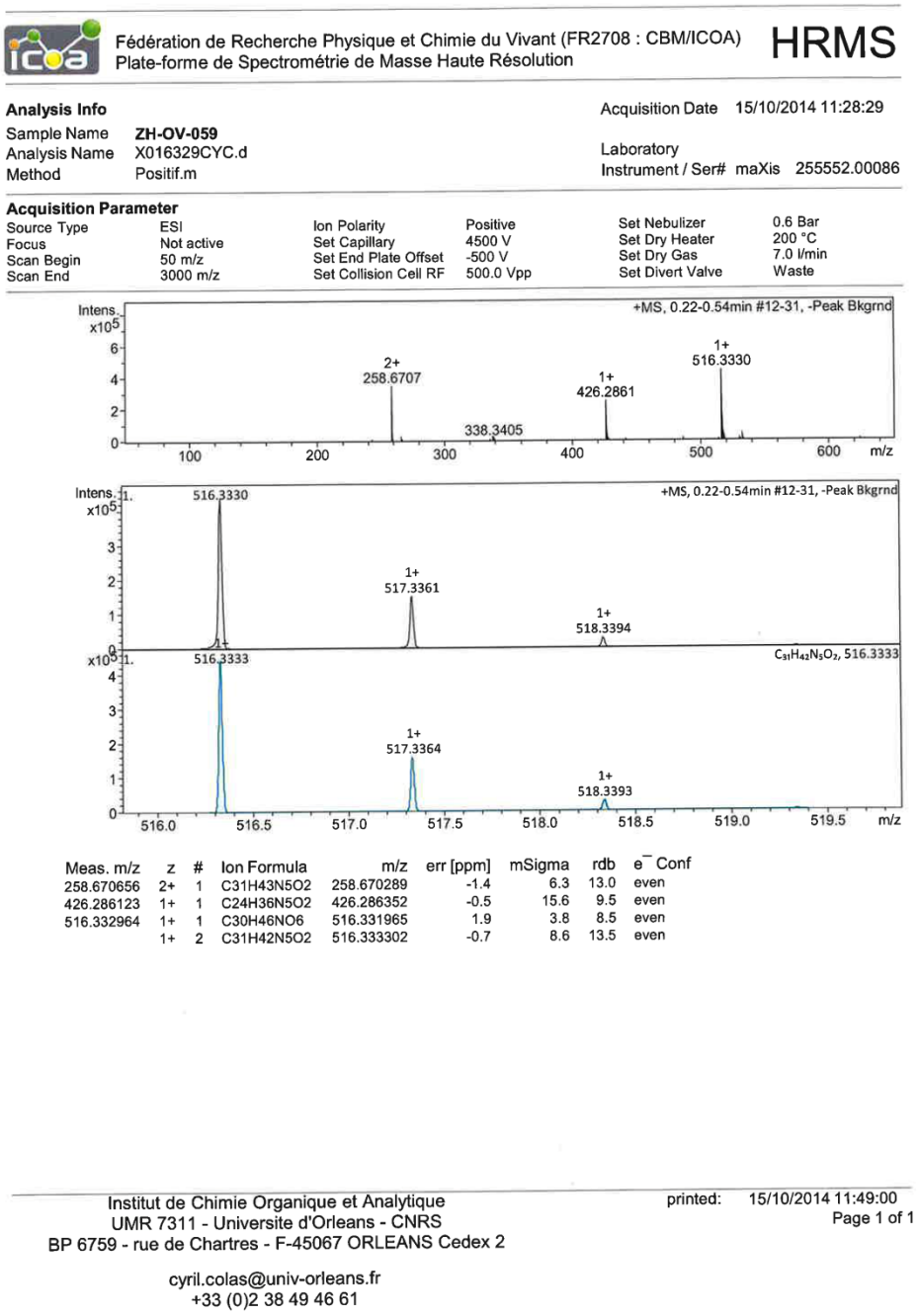
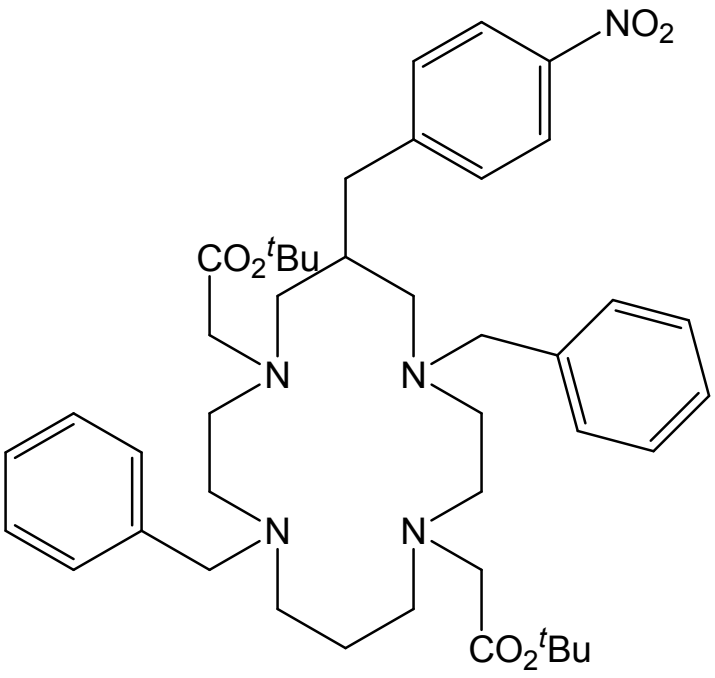


Figure S9. HRMS spectrum (ESI) of compound **6**

Compound 7:



Chemical Formula: $C_{43}H_{61}N_5O_6$
Exact Mass: 743,4622
Molecular Weight: 743,9743
m/z: 743.4622 (100.0%), 744.4655 (46.5%), 745.4689 (10.6%), 744.4592 (1.8%), 746.4722 (1.6%), 745.4664 (1.2%)

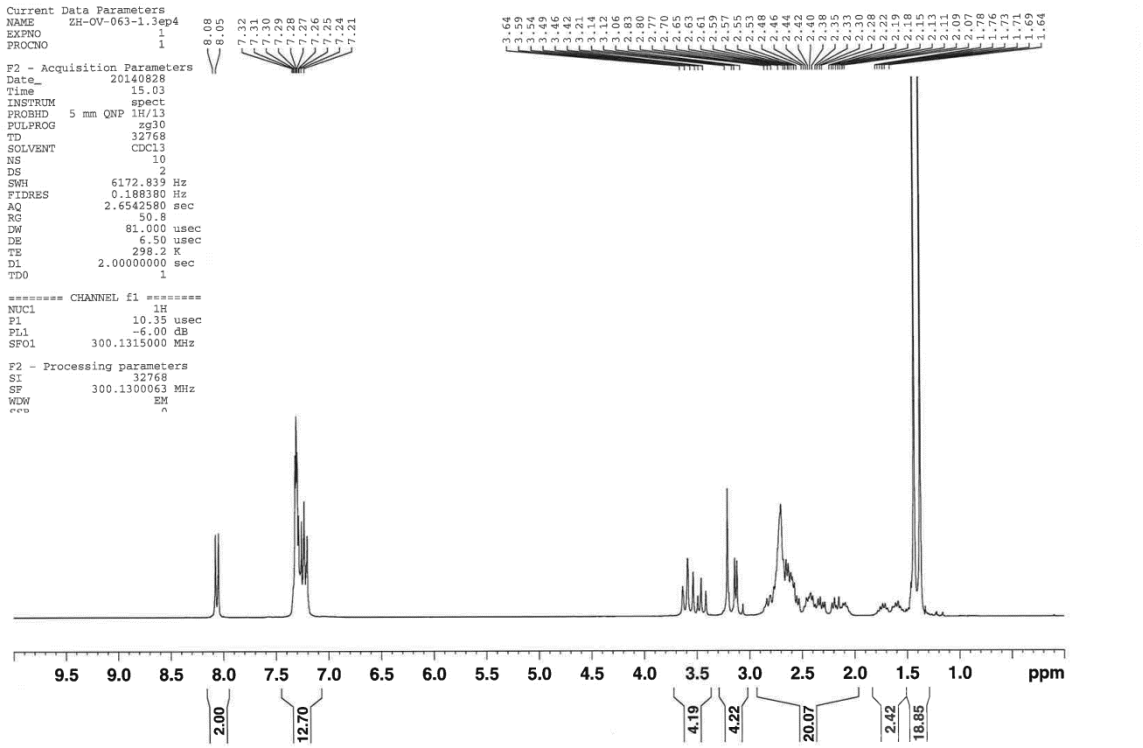


Figure S10. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **7**

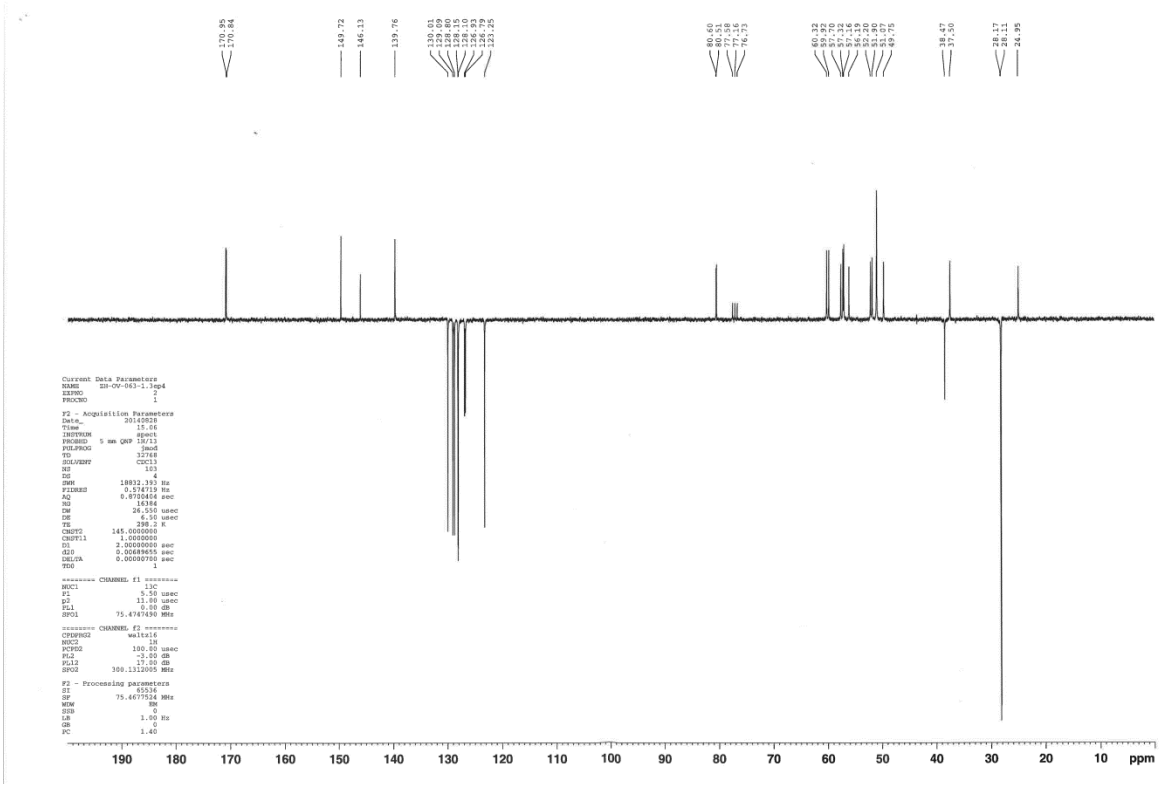


Figure S11. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **7**

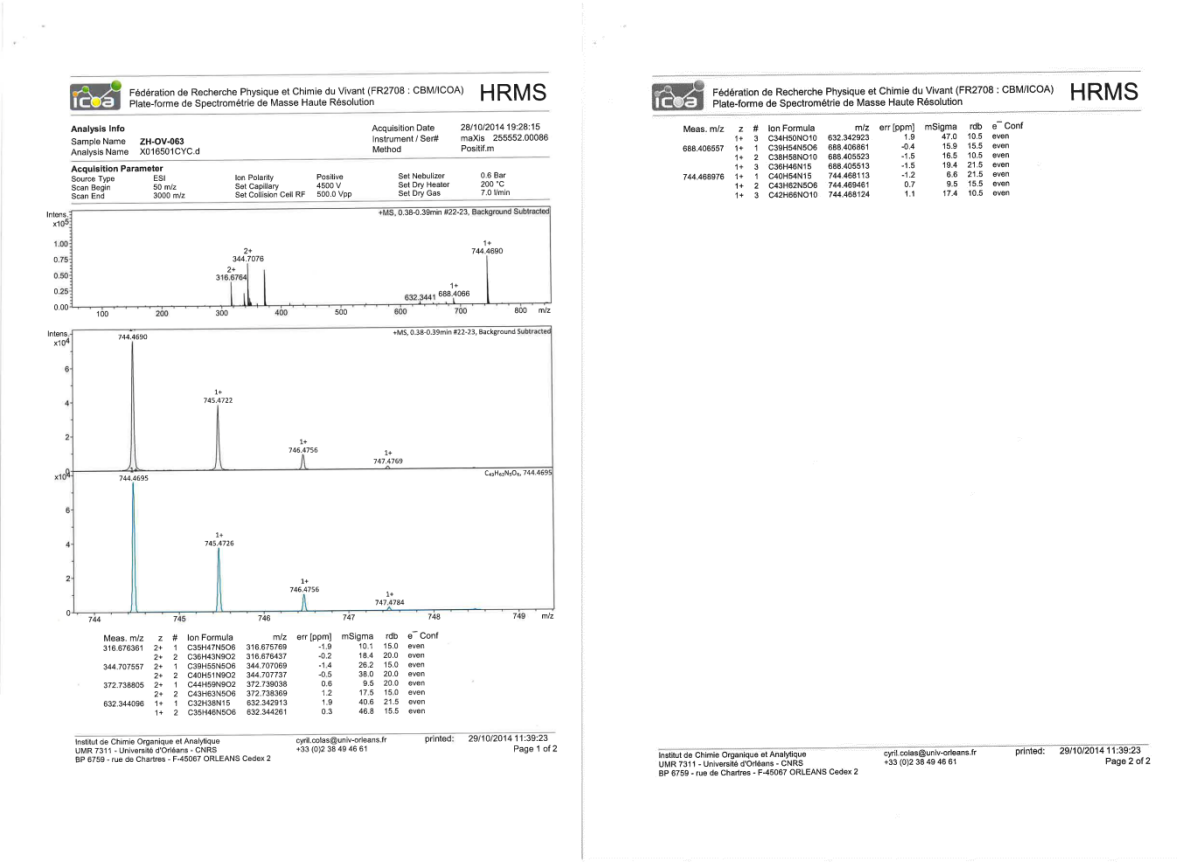
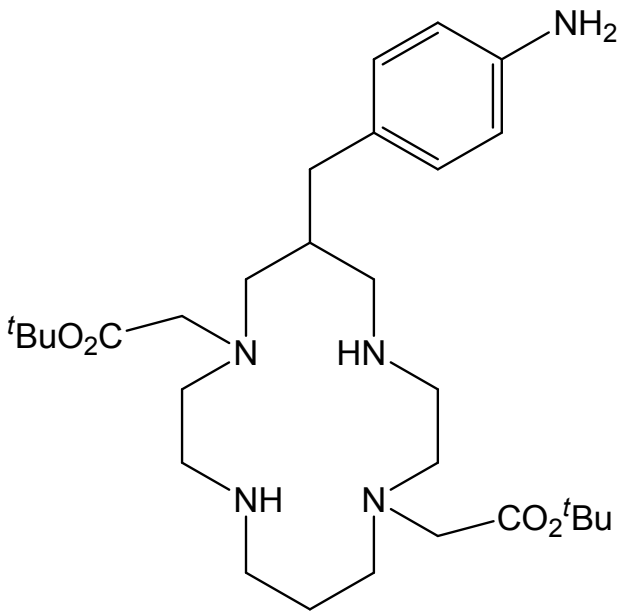


Figure S12. HRMS spectrum (ESI) of compound **7**

Compound 8:



Chemical Formula: $C_{29}H_{51}N_5O_4$
Exact Mass: 533,3941
Molecular Weight: 533,7463
m/z: 533.3941 (100.0%), 534.3975 (31.4%), 535.4008 (4.7%), 534.3911 (1.8%)

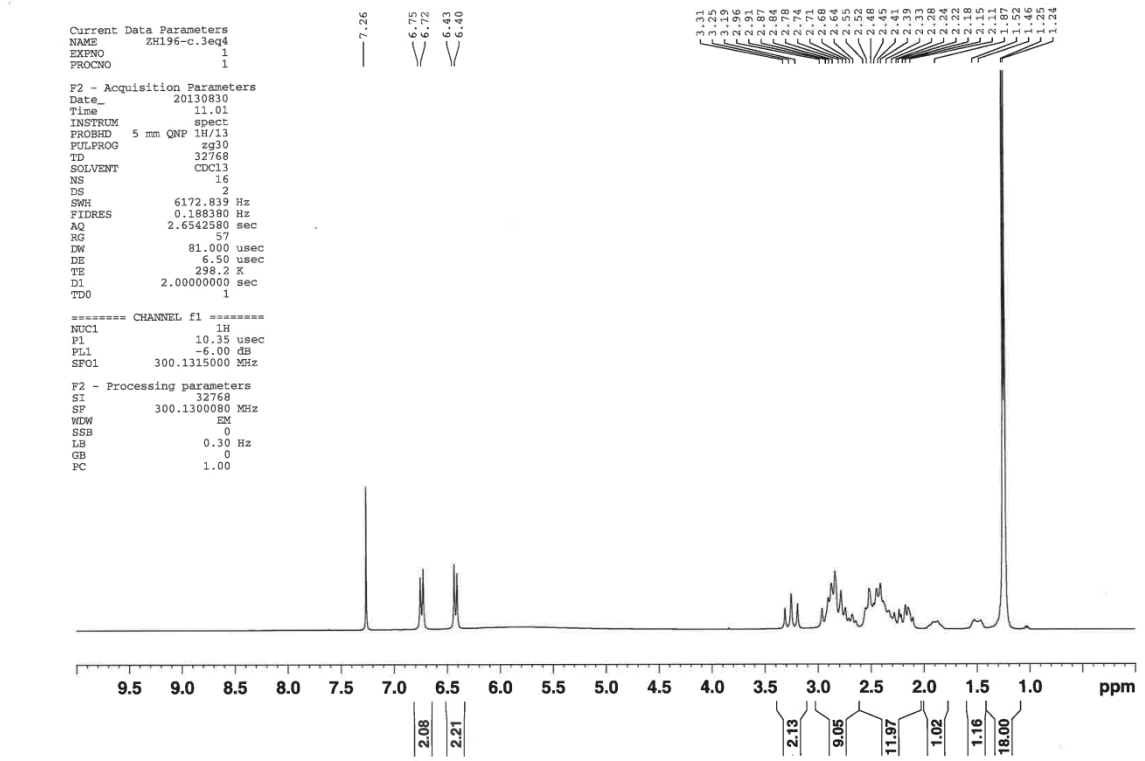


Figure S13. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **8**

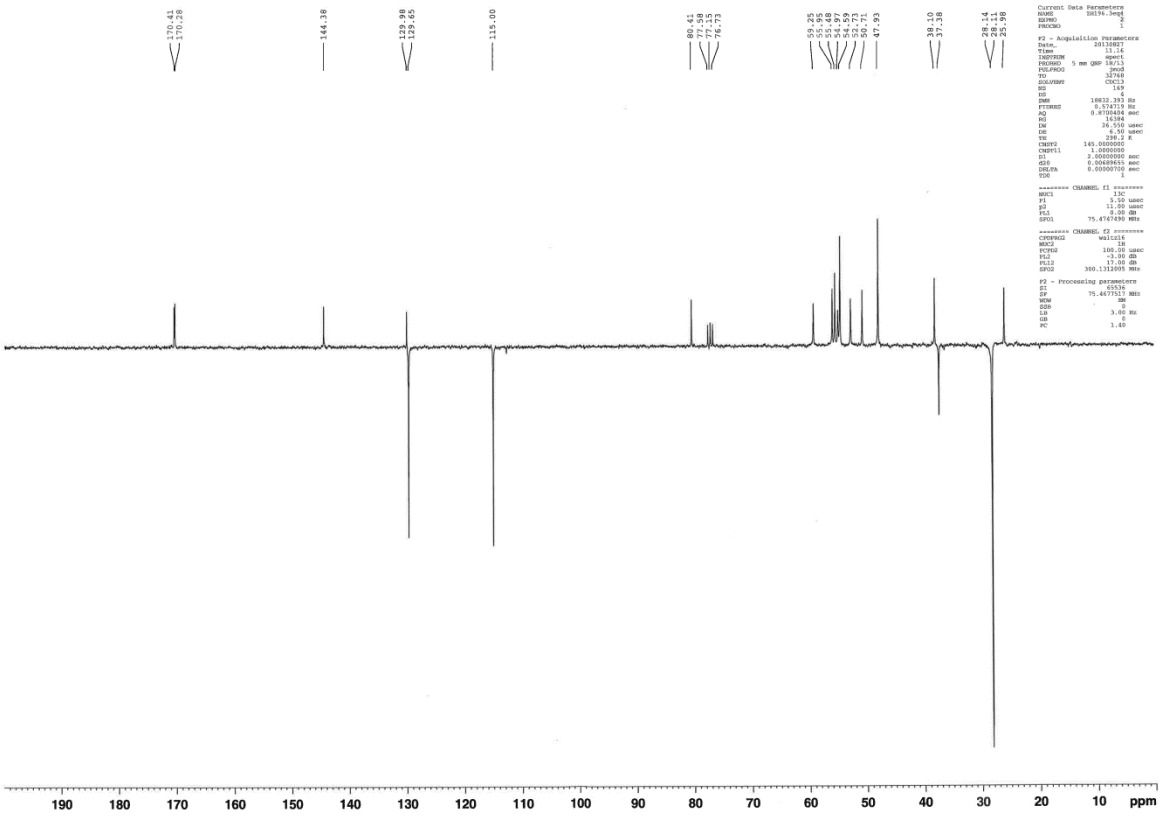


Figure S14. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **8**

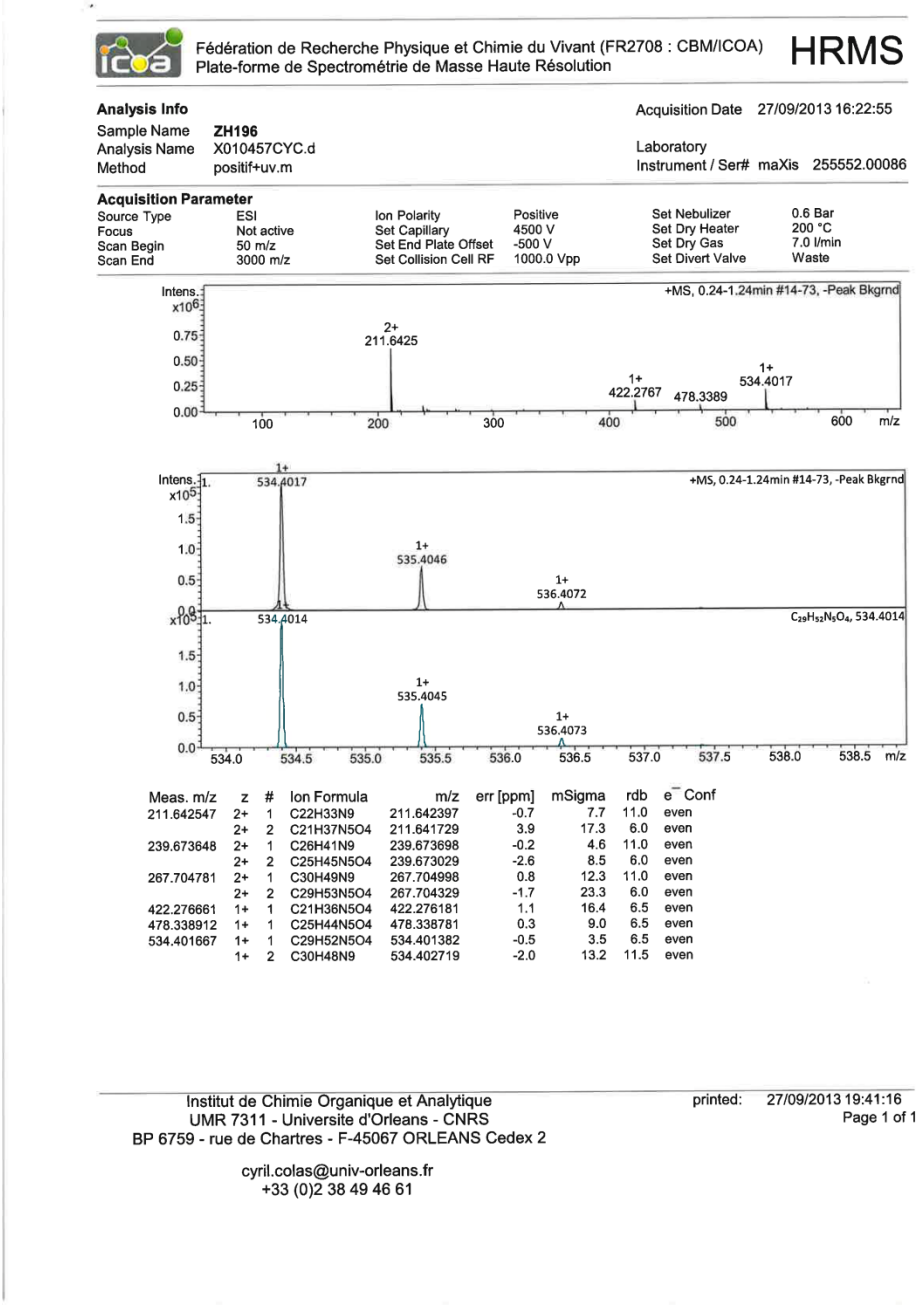
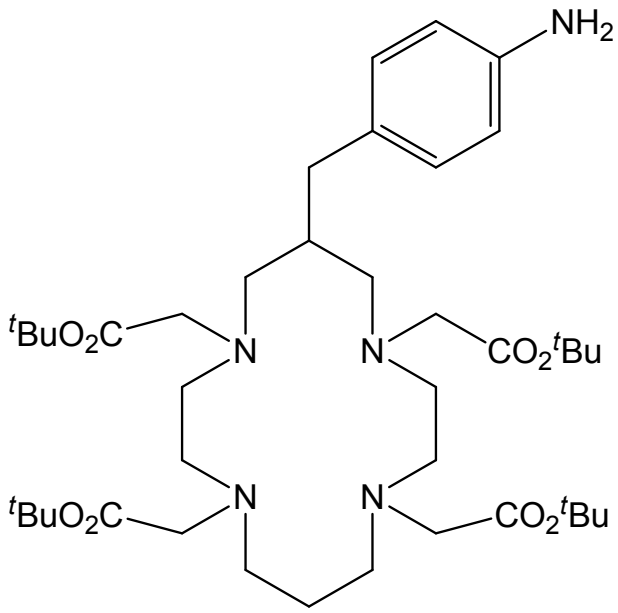


Figure S15. HRMS spectrum (ESI) of compound **8**

Compound 9:



Chemical Formula: $C_{41}H_{71}N_5O_8$
Exact Mass: 761,5303
Molecular Weight: 762,0311
m/z: 761.5303 (100.0%), 762.5336 (44.3%), 763.5370 (9.6%), 762.5273 (1.8%), 763.5345 (1.6%), 764.5403 (1.3%)

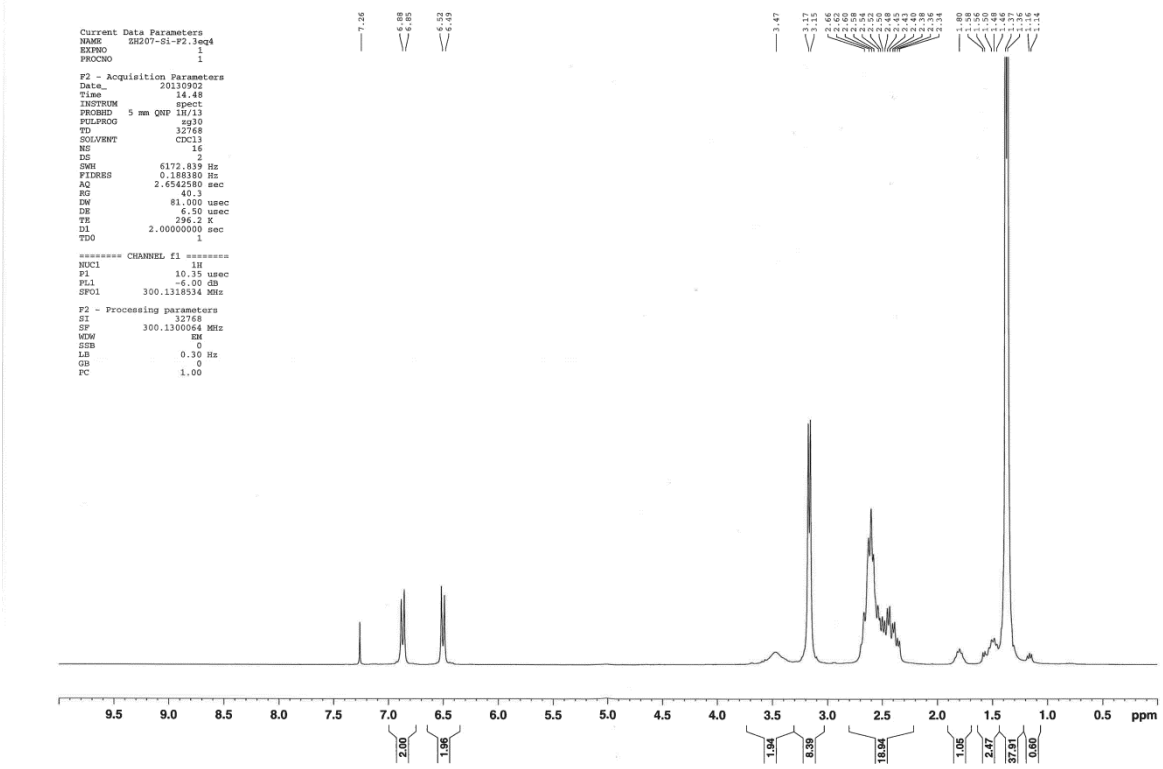


Figure S16. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **9**

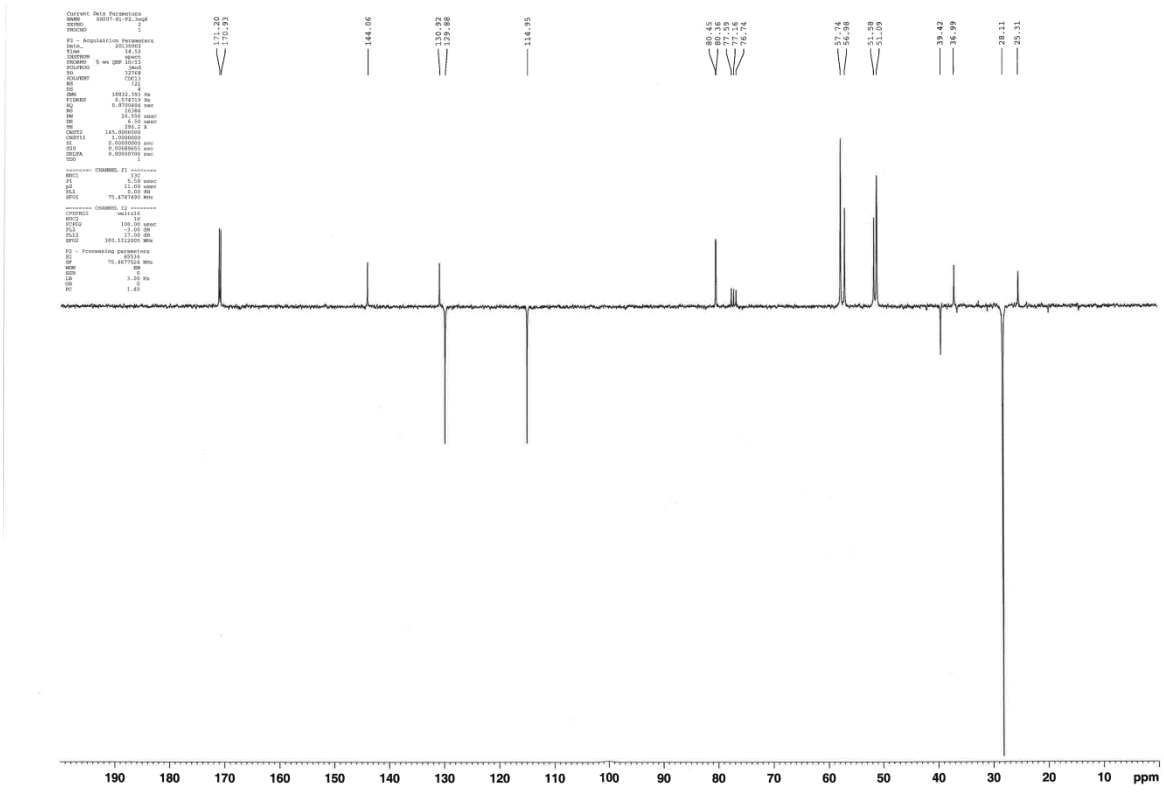


Figure S17. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **9**

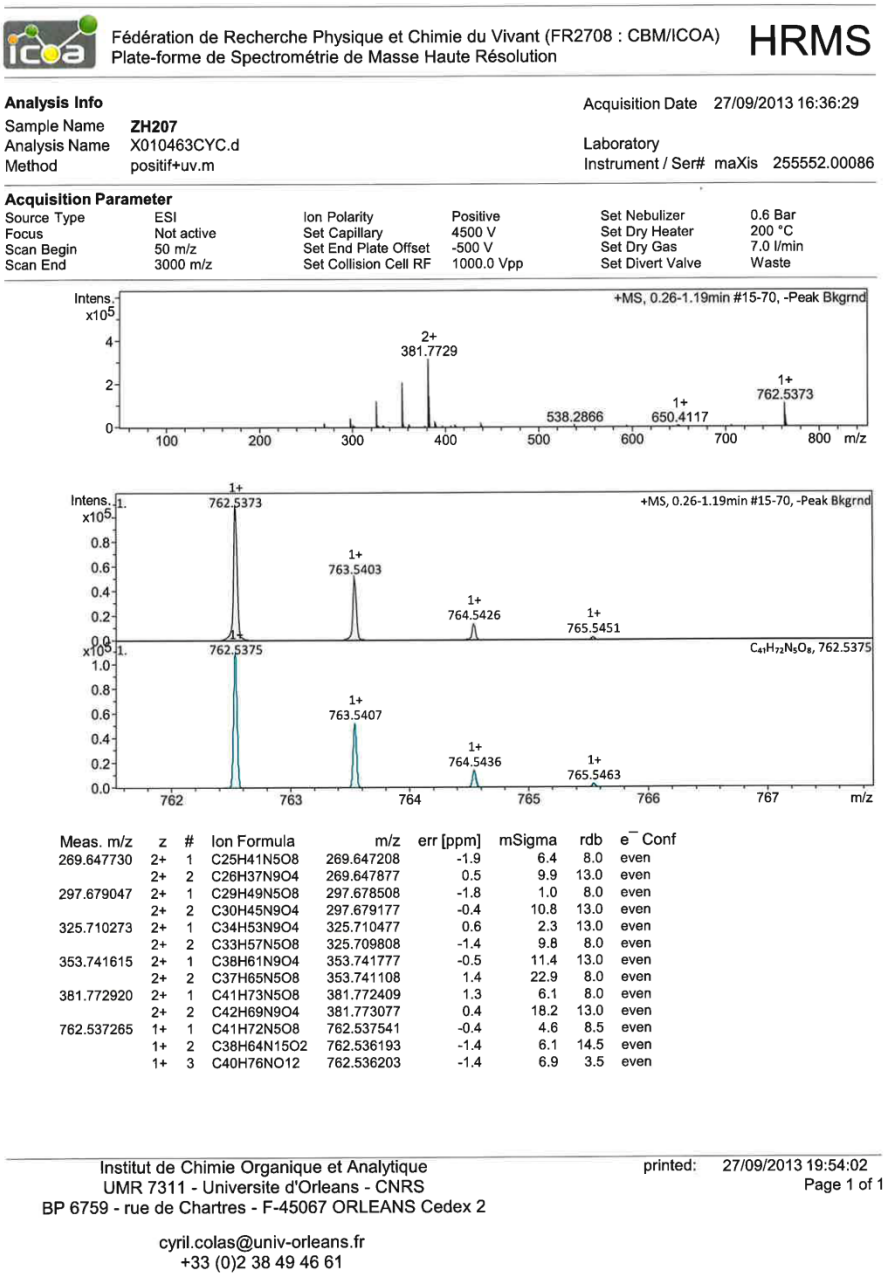
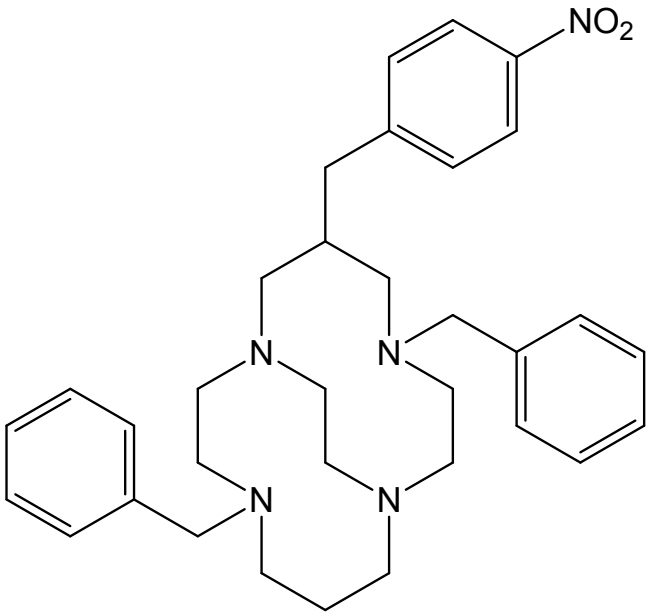


Figure S18. HRMS spectrum (ESI) of compound **9**

Compound 10:



Chemical Formula: $C_{33}H_{43}N_5O_2$
Exact Mass: 541,3417
Molecular Weight: 541,7268
m/z: 541.3417 (100.0%), 542.3450 (35.7%), 543.3484 (6.2%), 542.3387 (1.8%)

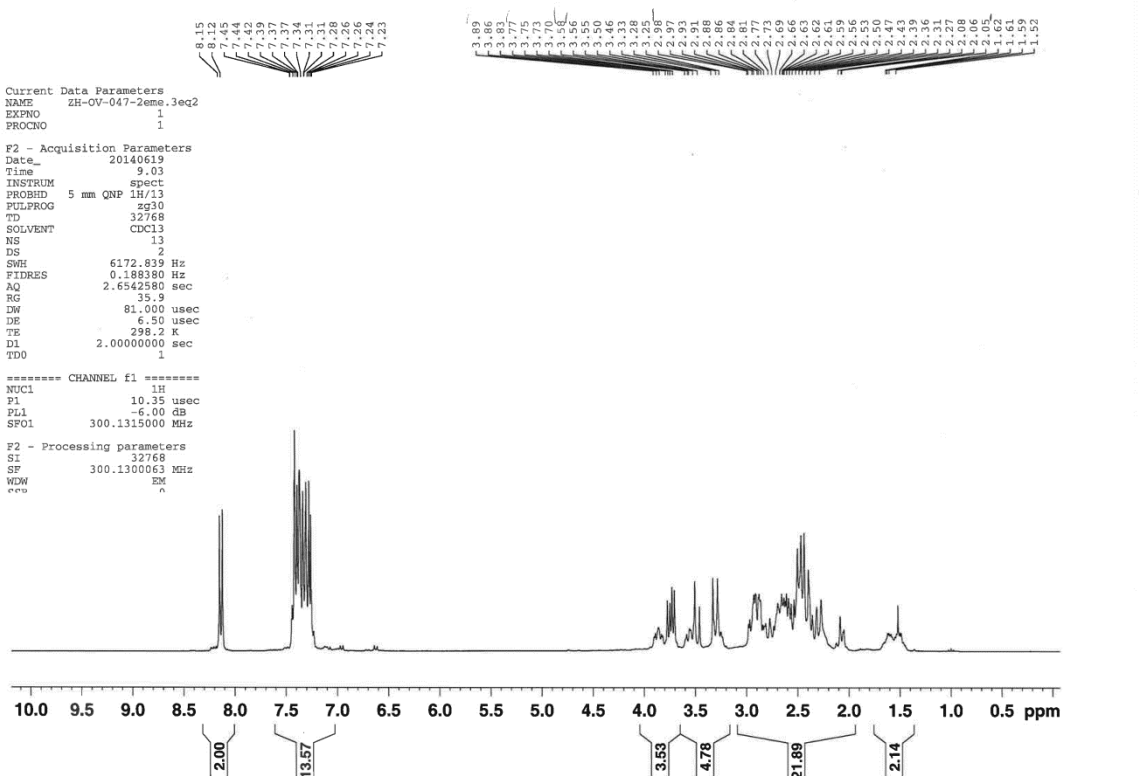


Figure S19. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **10**

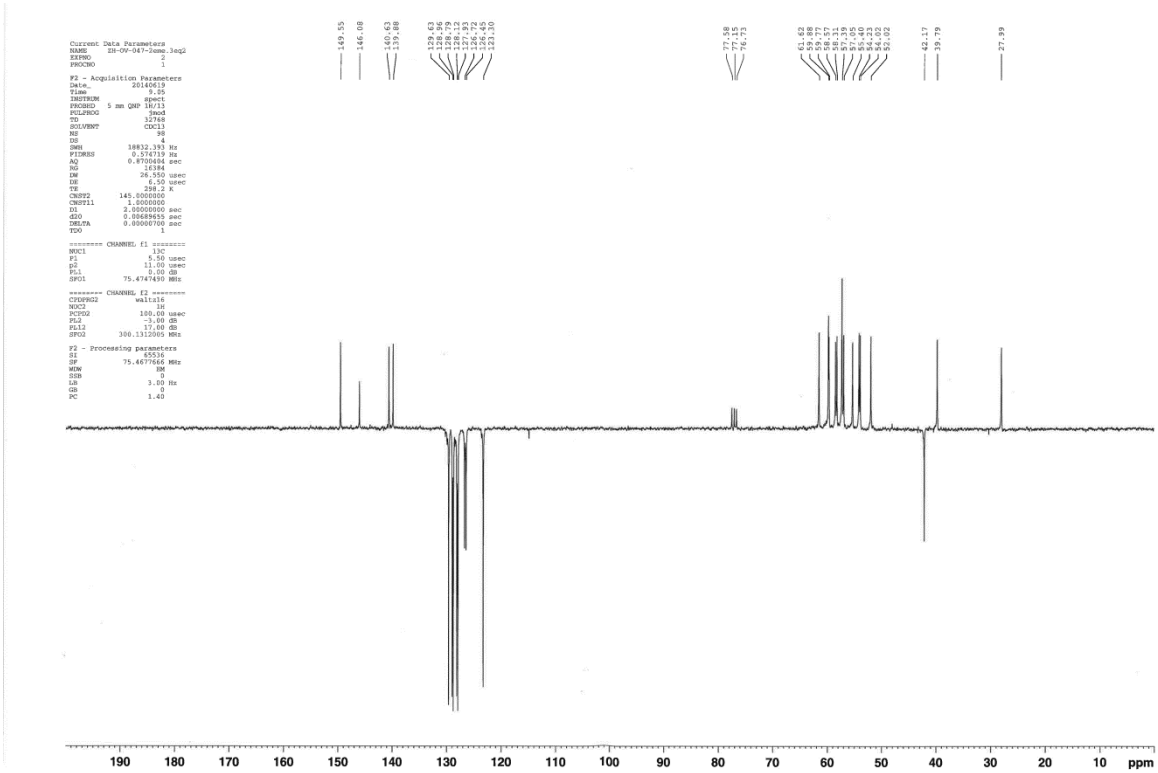


Figure S20. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **10**

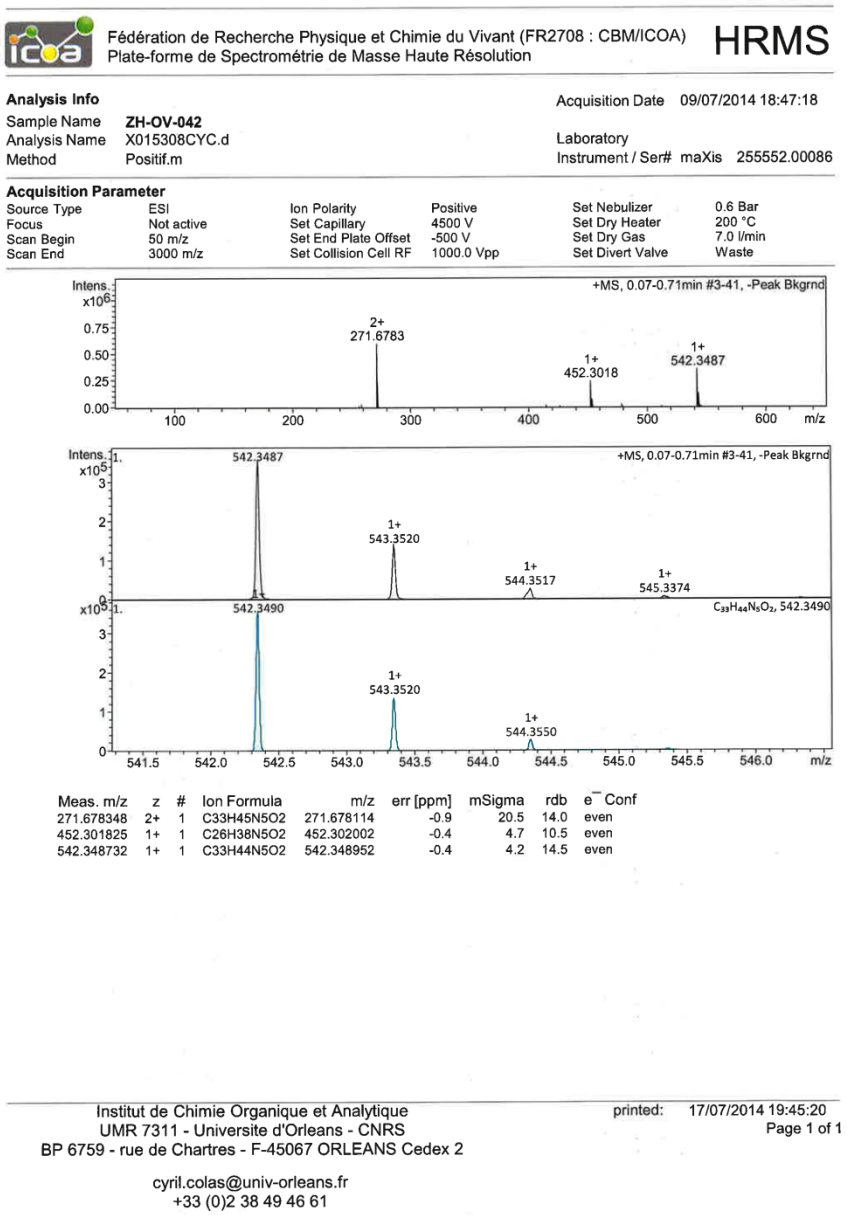
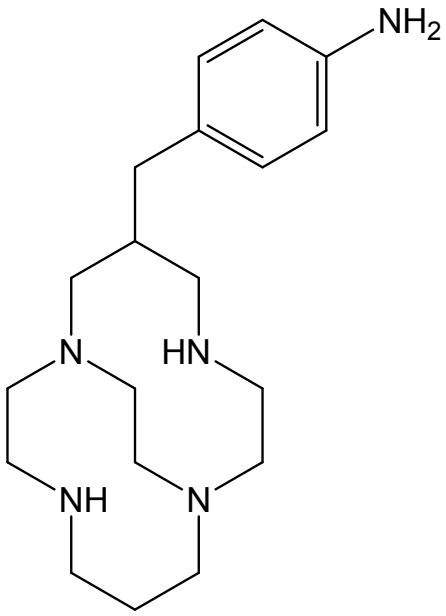


Figure S21. HRMS spectrum (ESI) of compound **10**

Compound 11:



Chemical Formula: $C_{19}H_{33}N_5$
Exact Mass: 331,2736
Molecular Weight: 331,4988
m/z: 331.2736 (100.0%), 332.2770 (20.5%), 333.2803 (2.0%), 332.2706 (1.8%)

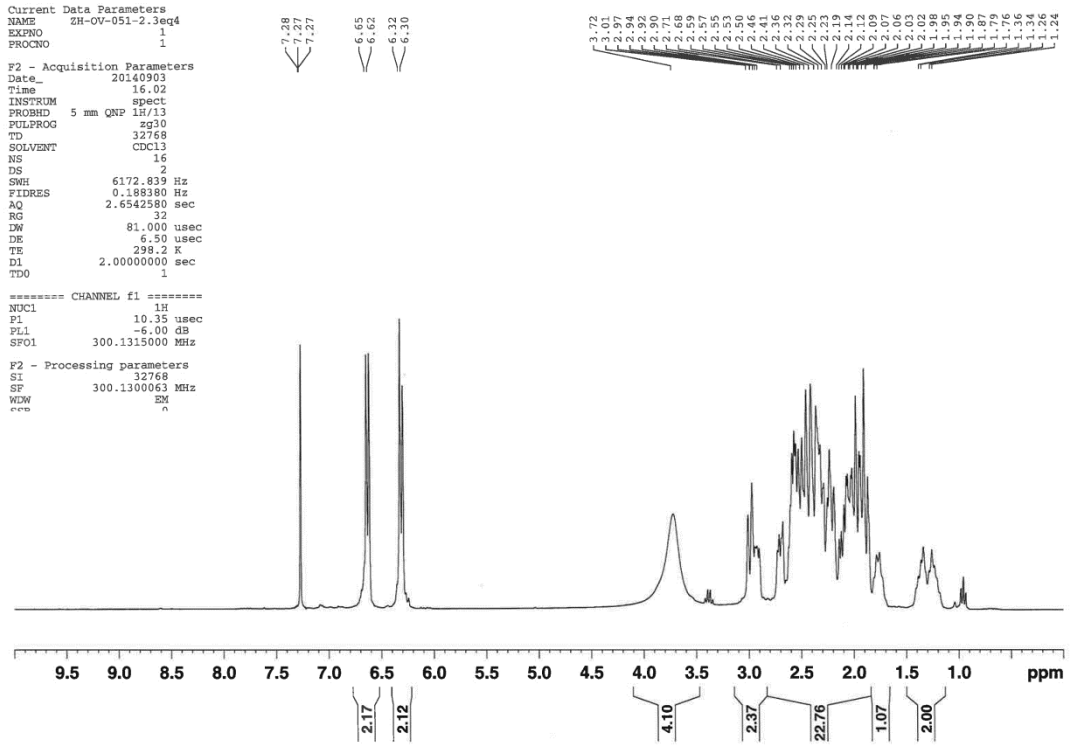


Figure S22. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **11**

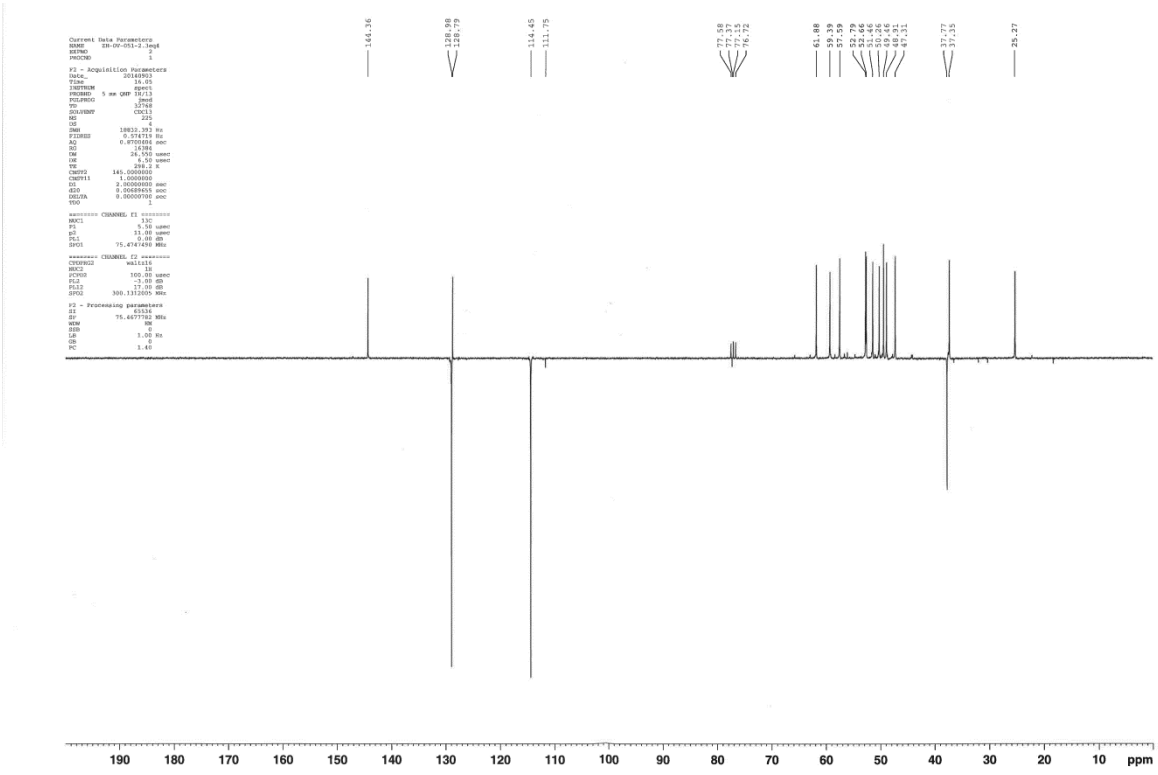


Figure S23. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **11**

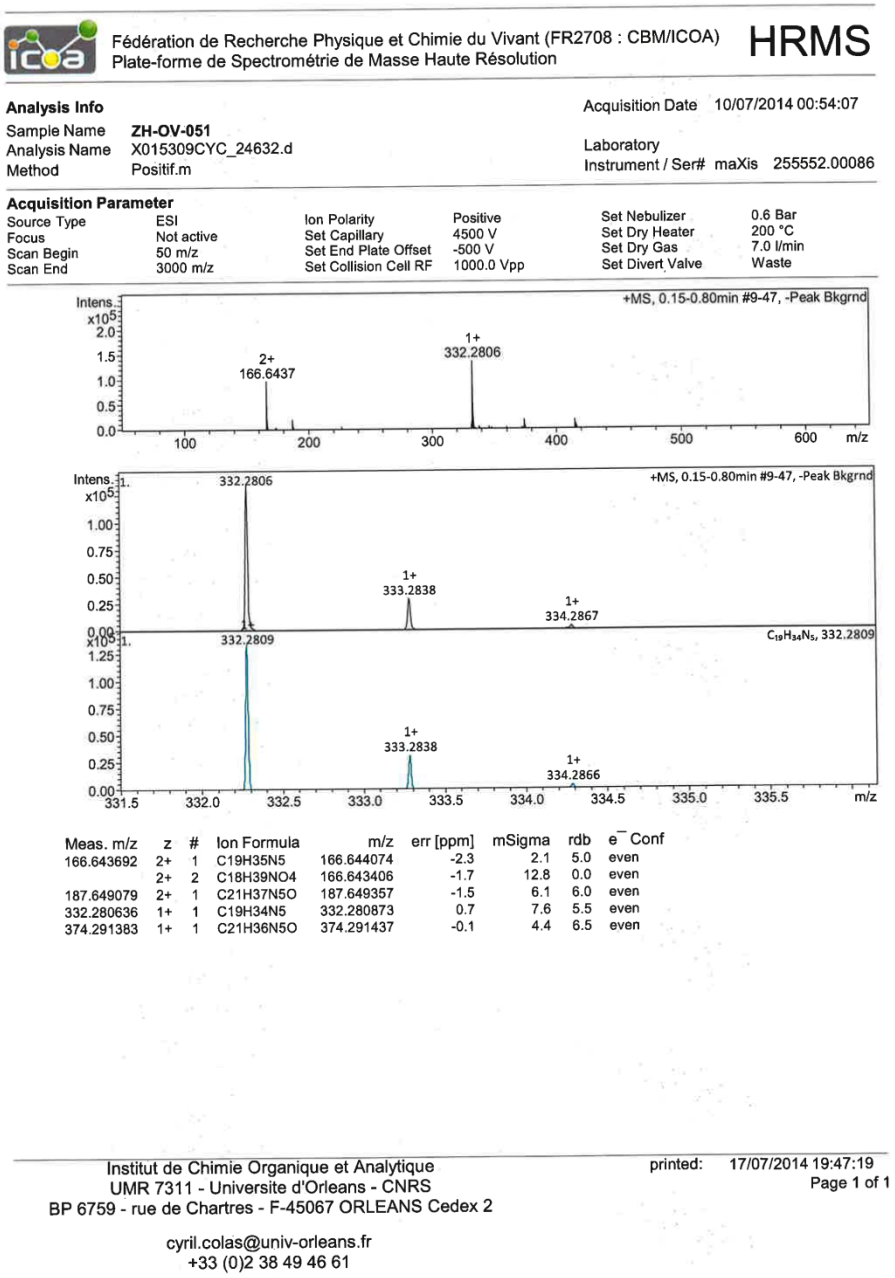
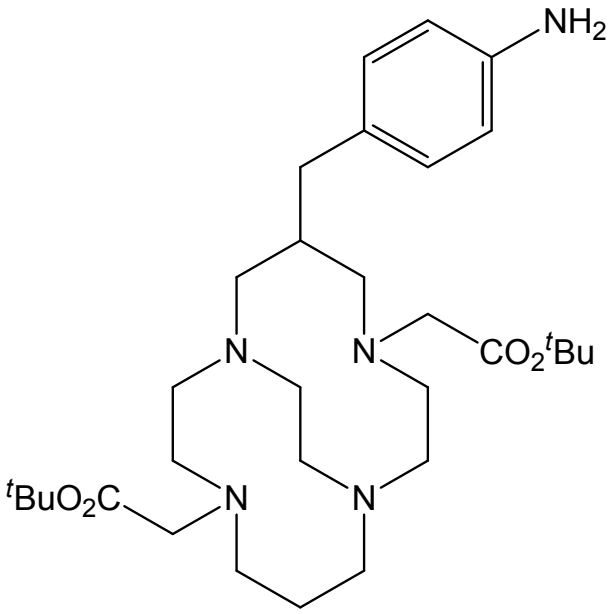


Figure S24. HRMS spectrum (ESI) of compound **11**

Compound 12:



Chemical Formula: $C_{31}H_{53}N_5O_4$
Exact Mass: 559,4098
Molecular Weight: 559,7836
m/z: 559.4098 (100.0%), 560.4131 (33.5%), 561.4165 (5.4%), 560.4068 (1.8%)

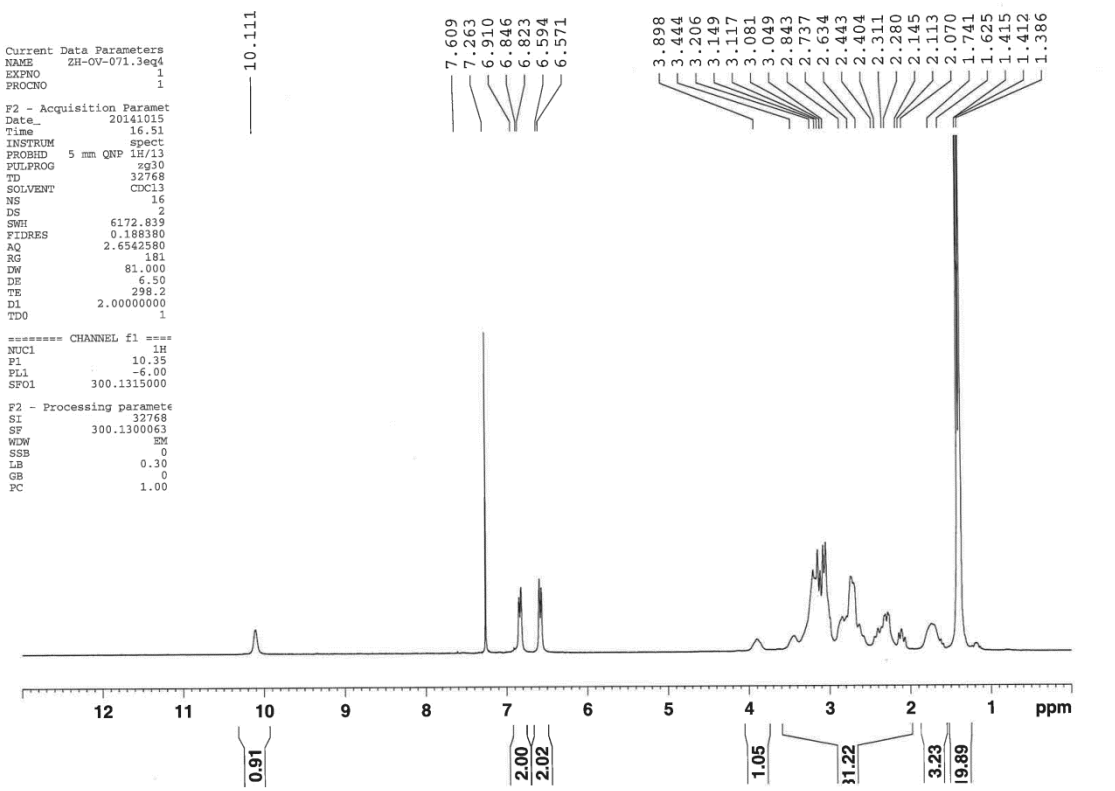


Figure S25. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **12**

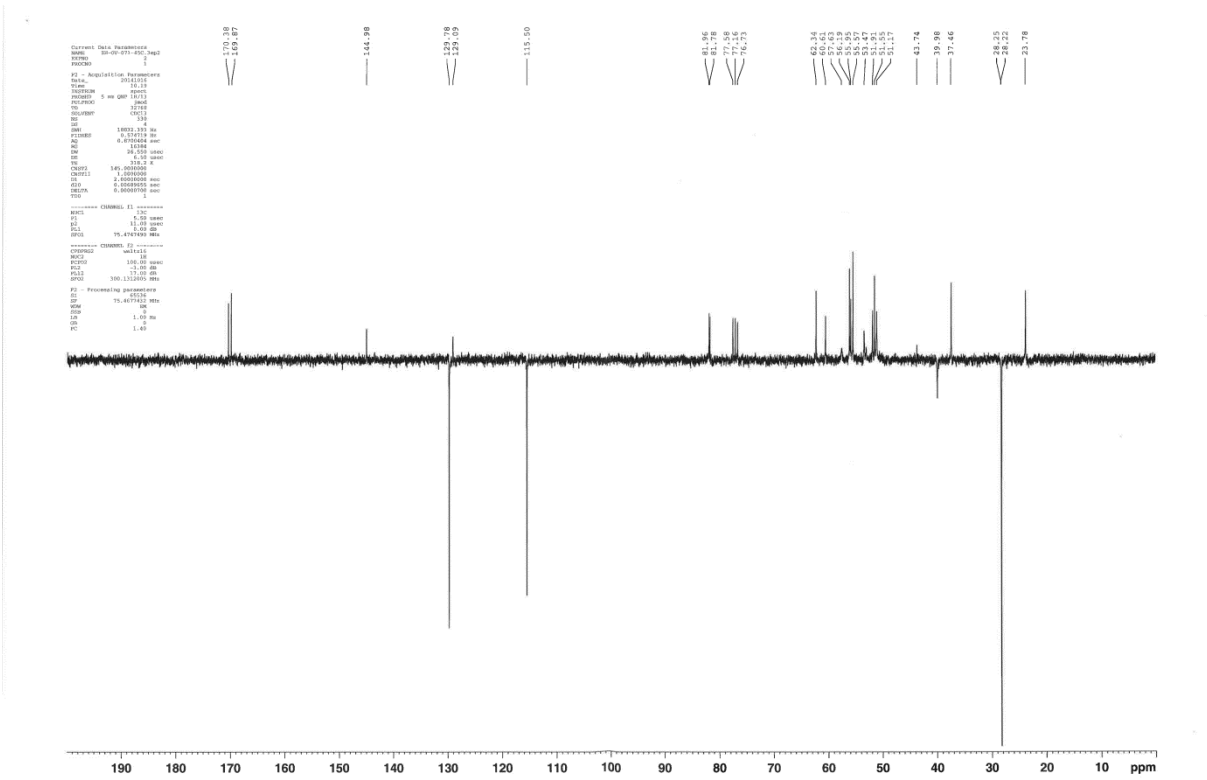


Figure S26. ^{13}C NMR spectrum (300 MHz, $CDCl_3$, 25 °C) of compound **12**

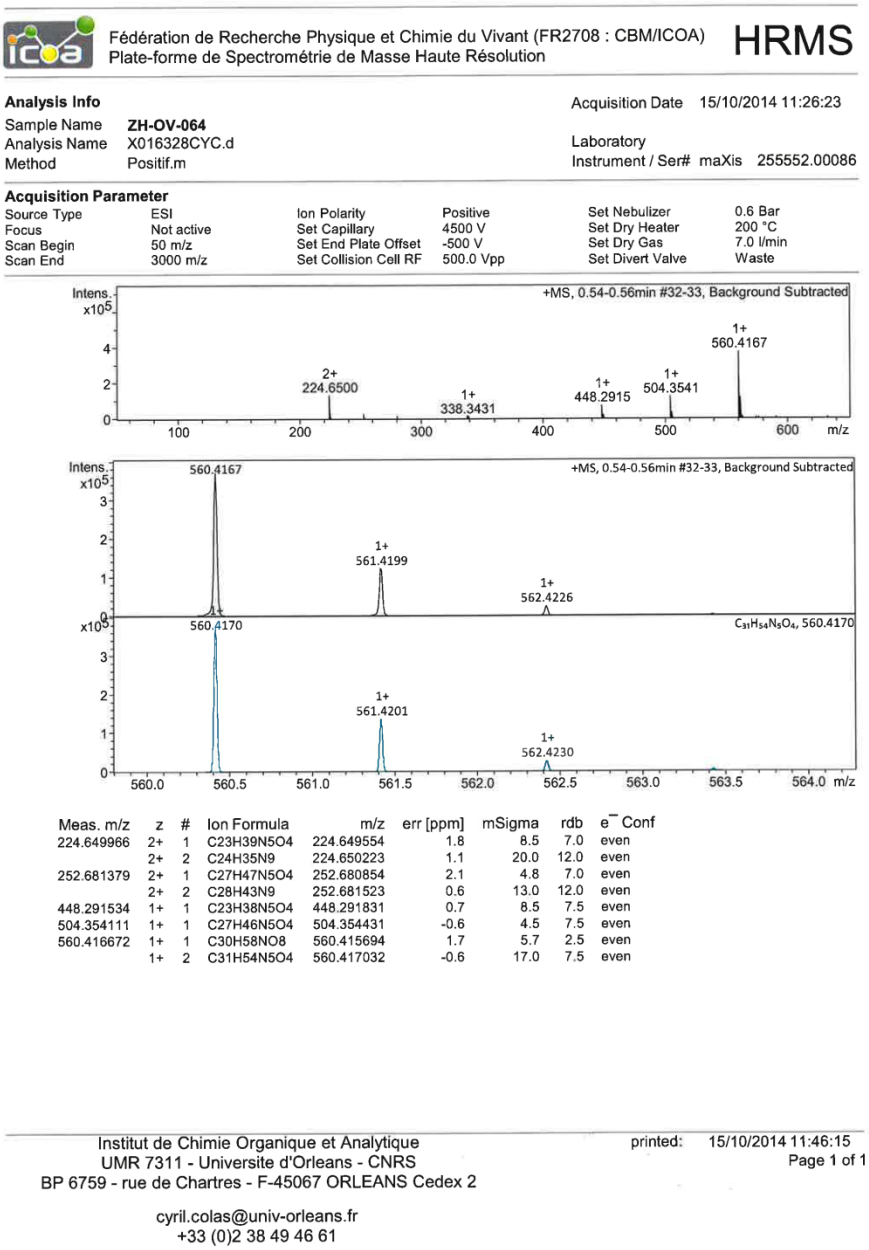
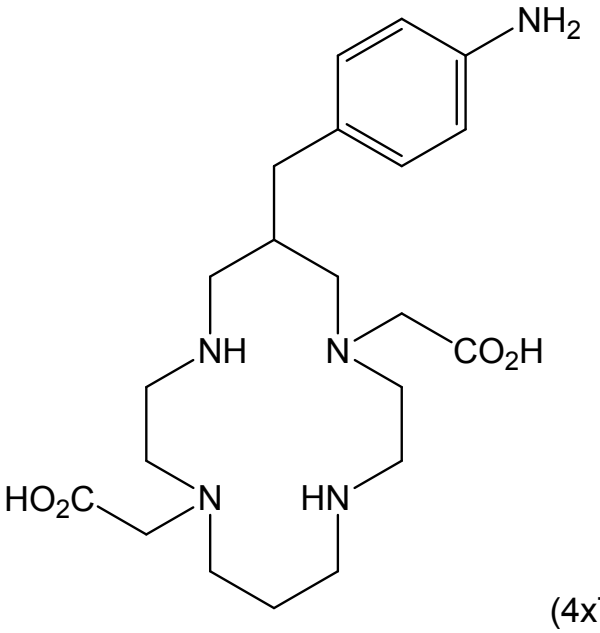


Figure S27. HRMS spectrum (ESI) of compound **12**

Compound 13:



(4xTFA)

Chemical Formula: $C_{21}H_{35}N_5O_4$
Exact Mass: 421,2689
Molecular Weight: 421,5337
m/z: 421.2689 (100.0%), 422.2723 (22.7%), 423.2756 (2.5%), 422.2659 (1.8%)

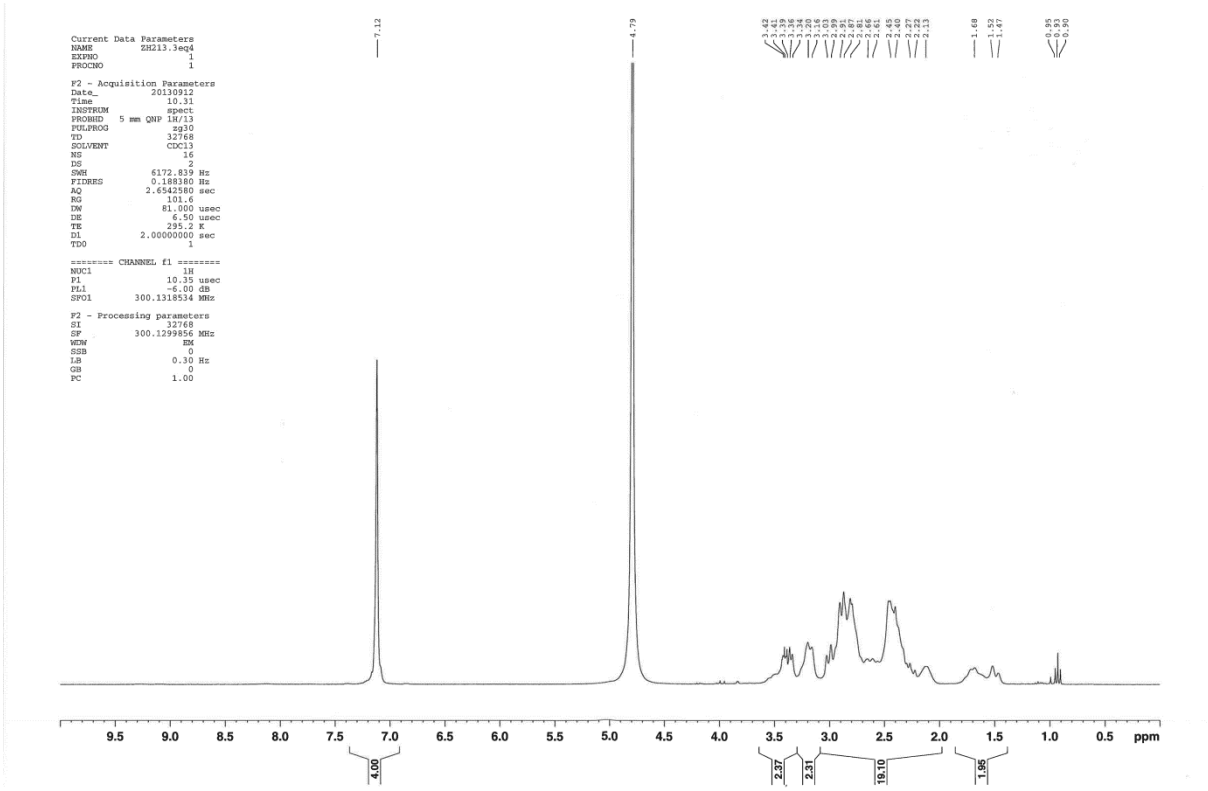


Figure S28. ^{1}H NMR spectrum (300 MHz, D_2O, 25 °C) of compound **13**

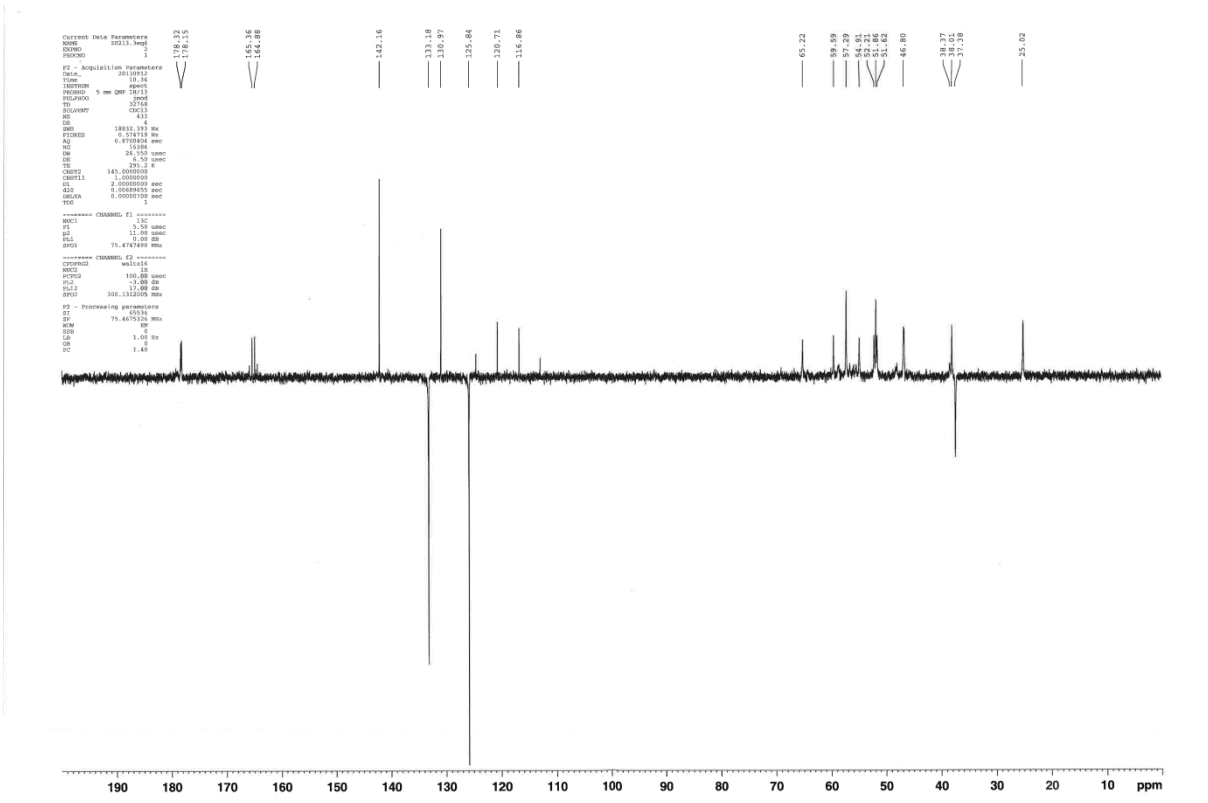
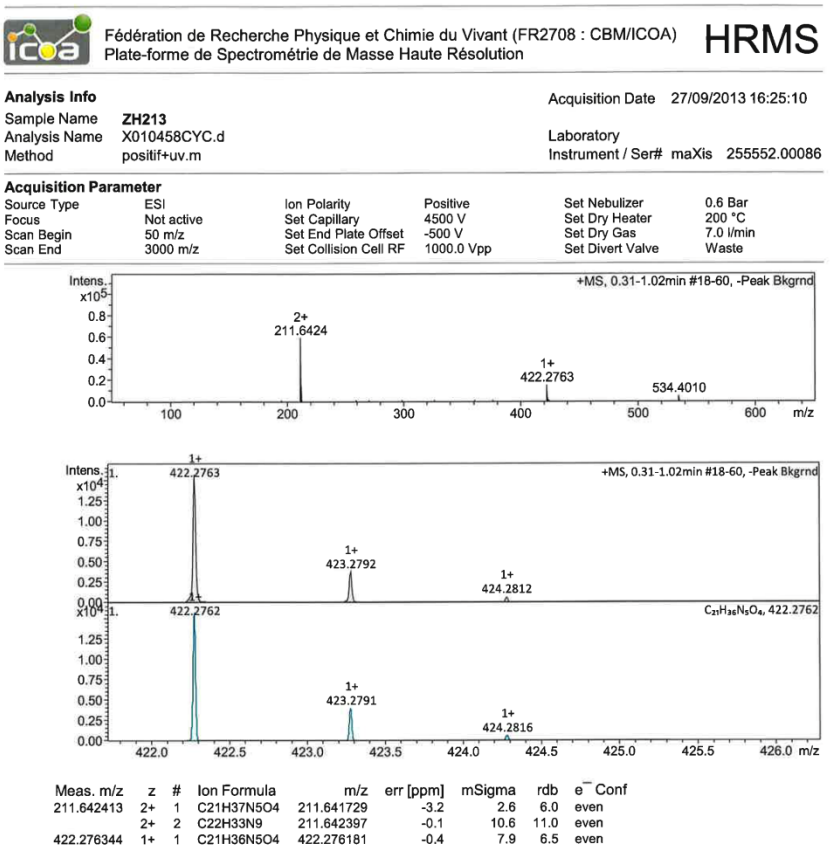


Figure S29. ^{13}C NMR spectrum (300 MHz, D_2O, 25 °C) of compound **13**



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
211.642413	2+	1	C21H37N5O4	211.641729	-3.2	2.6	6.0	even
	2+	2	C22H33N9	211.642397	-0.1	10.6	11.0	even
422.276344	1+	1	C21H36N5O4	422.276181	-0.4	7.9	6.5	even

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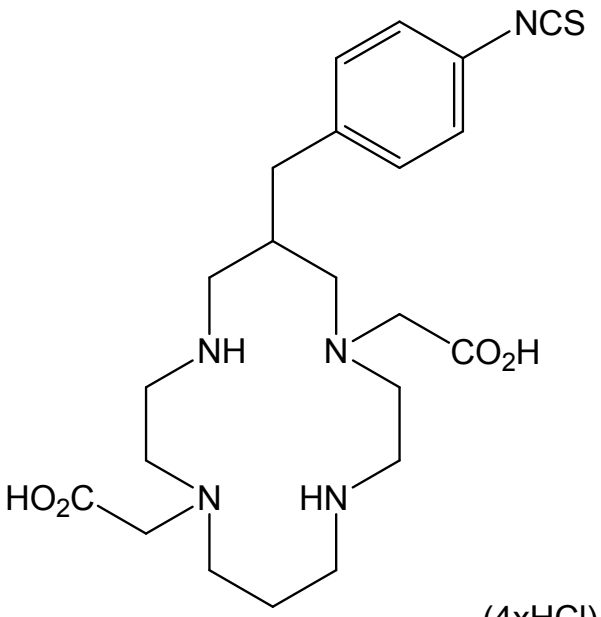
Figure S30. HRMS spectrum (ESI) of compound **13**

Compound 14:



(4xHCl)

Chemical Formula: $C_{22}H_{33}N_5O_4S$
Exact Mass: 463,2253
Molecular Weight: 463,5935
m/z: 463.2253 (100.0%), 464.2287 (23.8%), 465.2211 (4.5%), 465.2320 (2.7%), 464.2224 (1.8%), 466.2245 (1.1%)

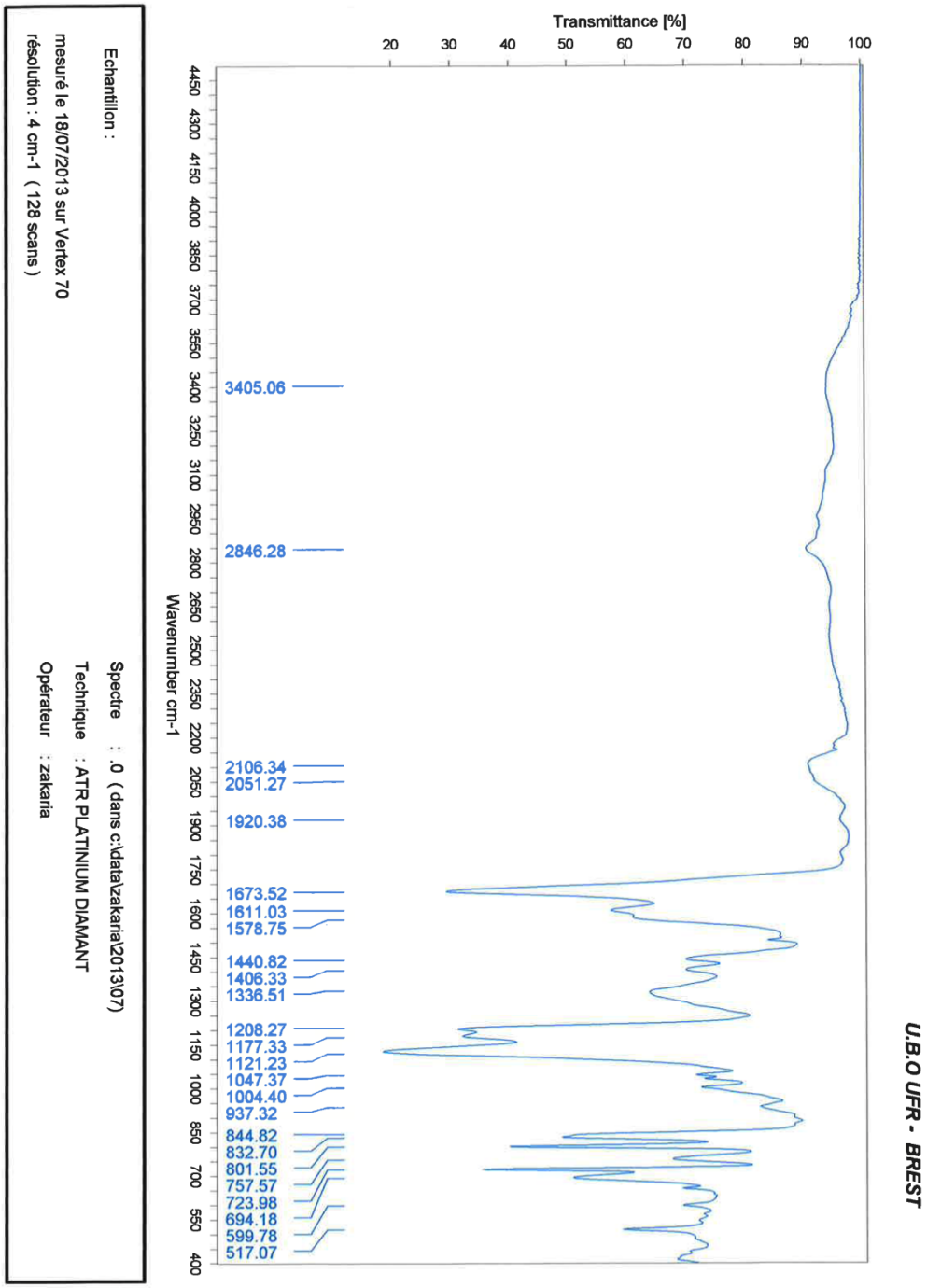


Figure S31. IR spectrum of compound **14**

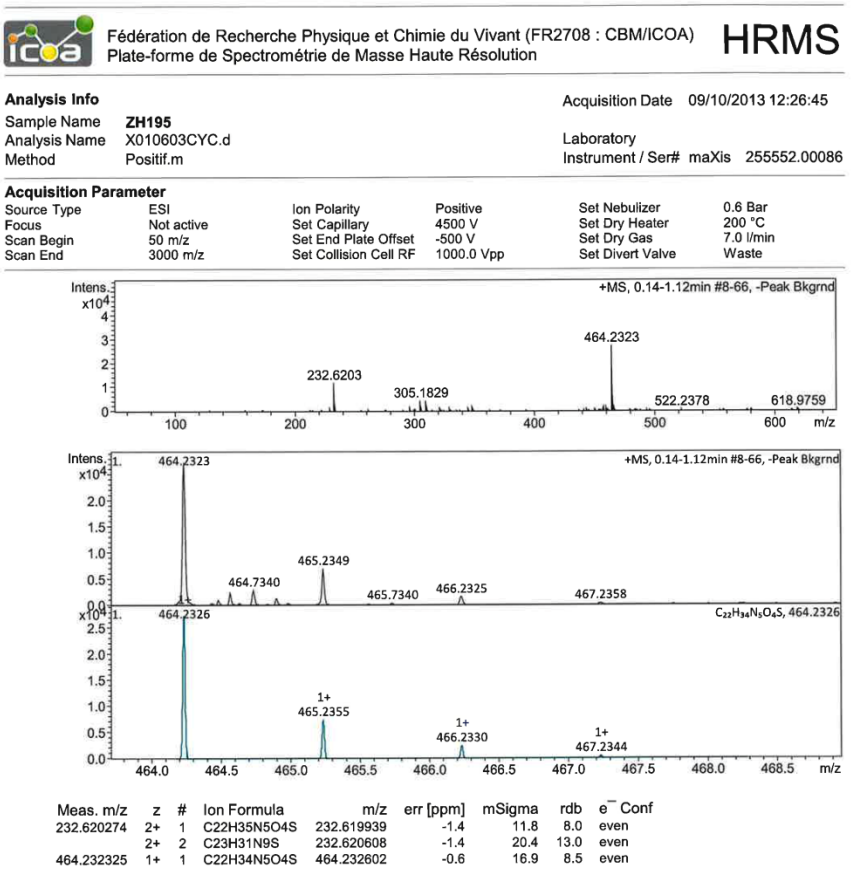


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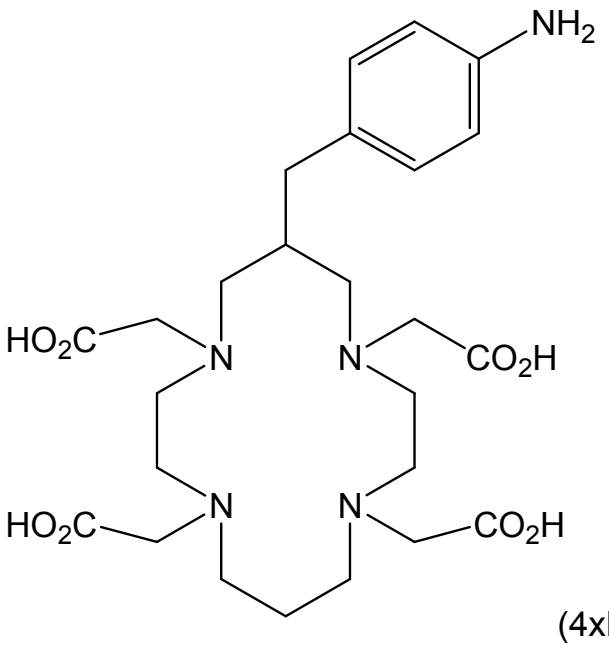
Figure S32. HRMS spectrum (ESI) of compound **14**

Compound 15:



(4xHCl)

$C_{25}H_{39}N_5O_8$
Exact Mass: 537,2799
Mol. Wt.: 537,6059
m/e: 537,2799 (100,0%), 538,2832 (27,0%), 539,2866 (3,5%), 538,2769 (1,8%), 539,2841 (1,6%)
C, 55.85; H, 7.31; N, 13.03; O, 23.81

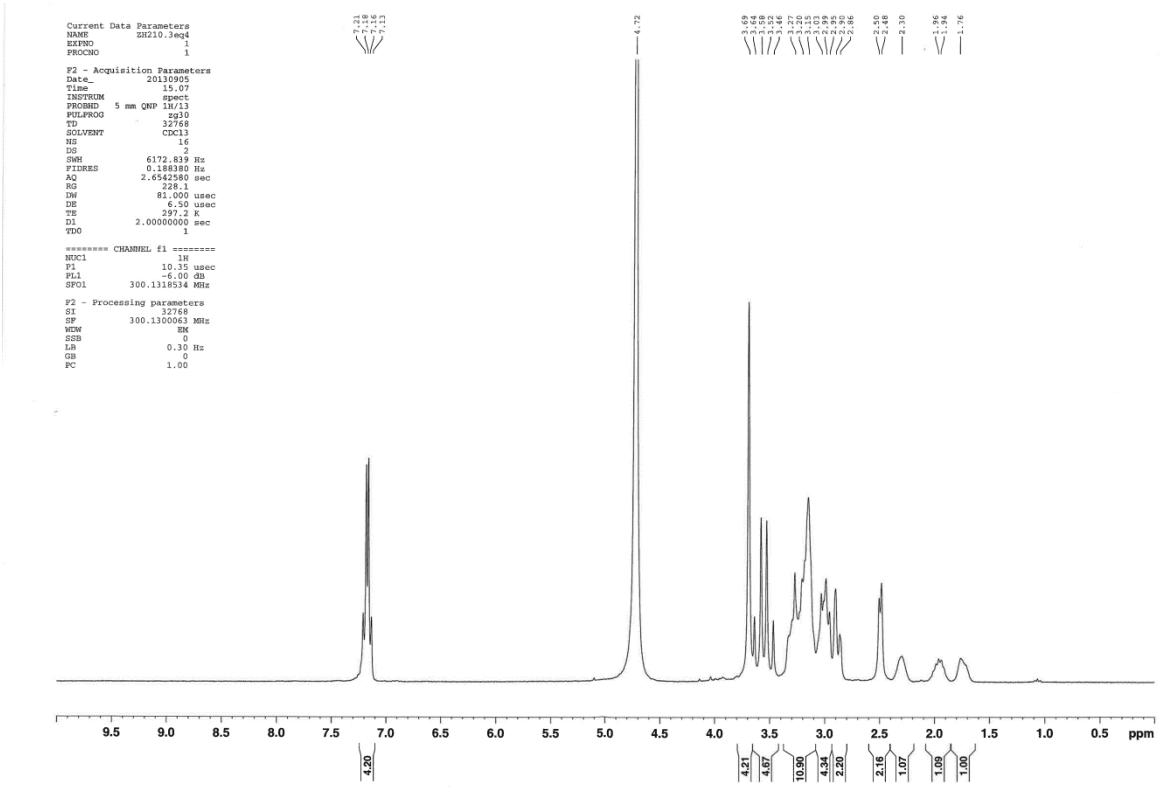


Figure S33. ^{1}H NMR spectrum (300 MHz, D_2O, 25 °C) of compound **15**

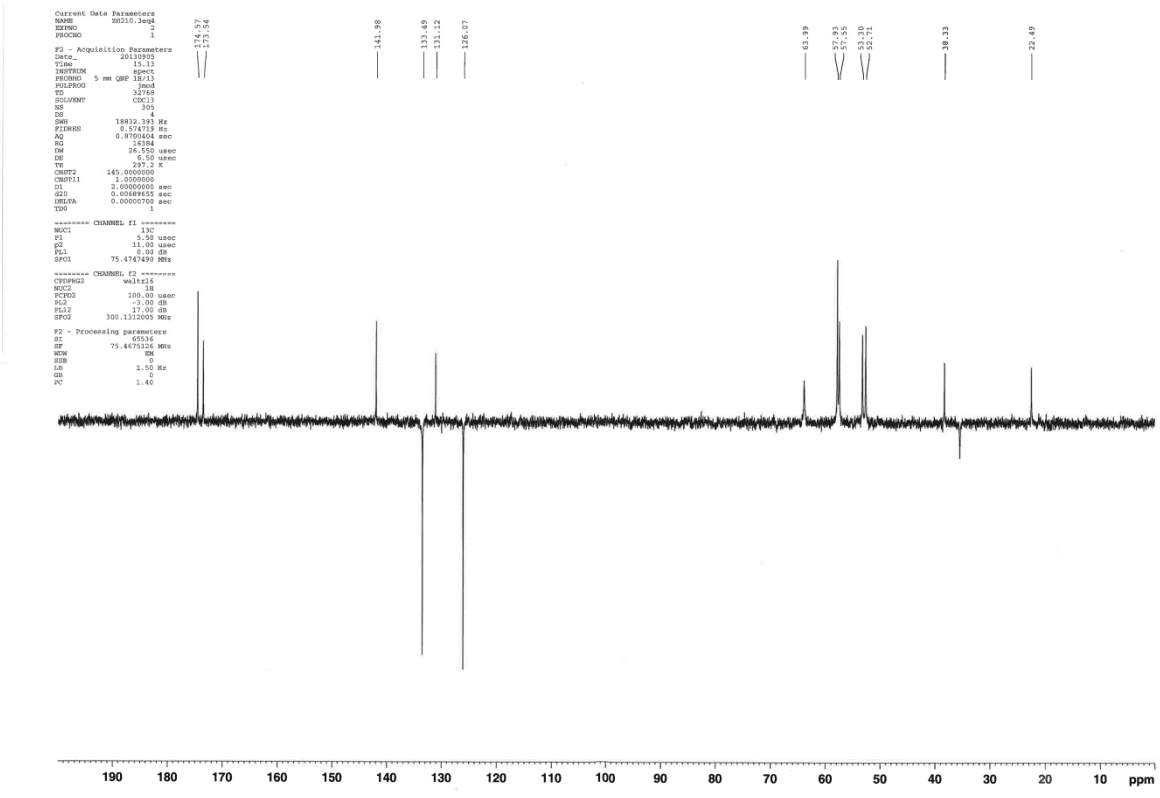


Figure S34. ^{13}C NMR spectrum (300 MHz, D_2O, 25 °C) of compound **15**

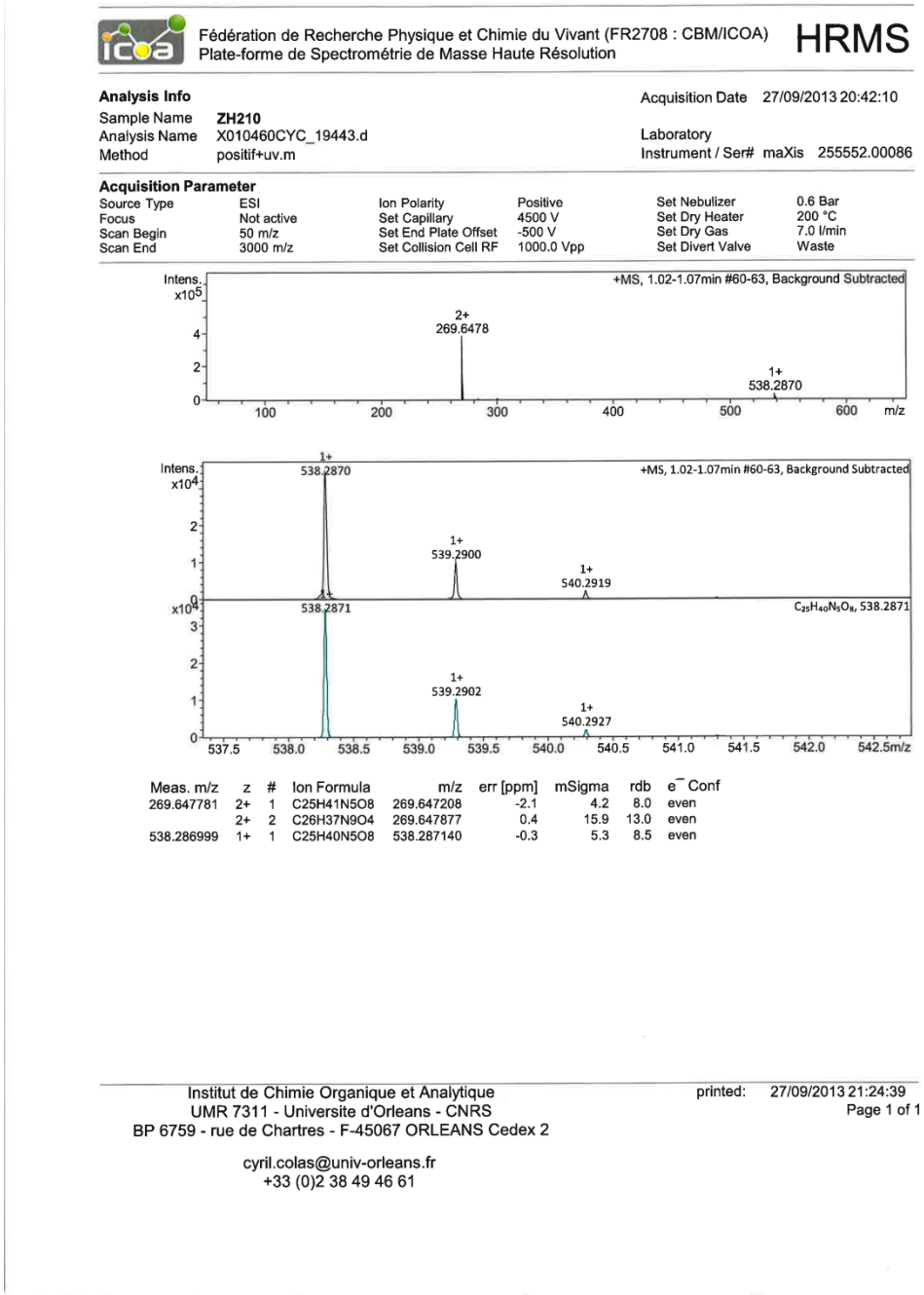
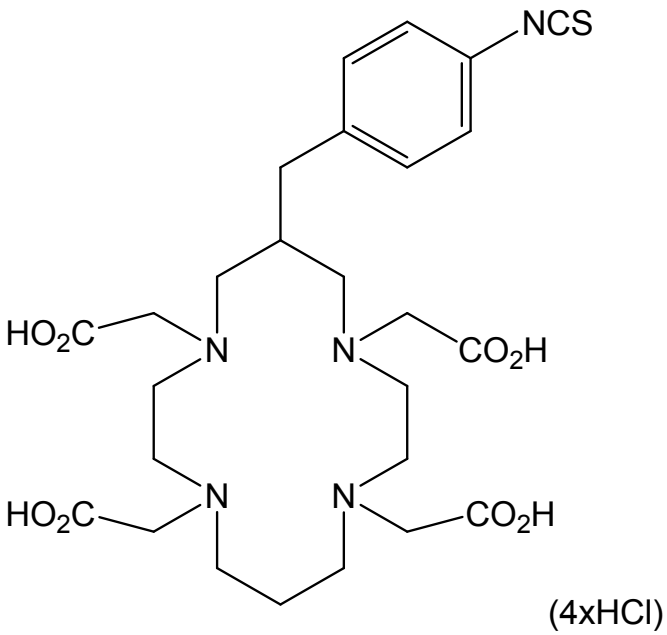


Figure S35. HRMS spectrum (ESI) of compound **15**

Compound 16:



Chemical Formula: $C_{26}H_{37}N_5O_8S$
Exact Mass: 579,2363
Molecular Weight: 579,6657
m/z: 579.2363 (100.0%), 580.2396 (28.1%), 581.2321 (4.5%), 581.2430 (3.8%), 580.2333 (1.8%), 581.2405 (1.6%), 582.2354 (1.3%)

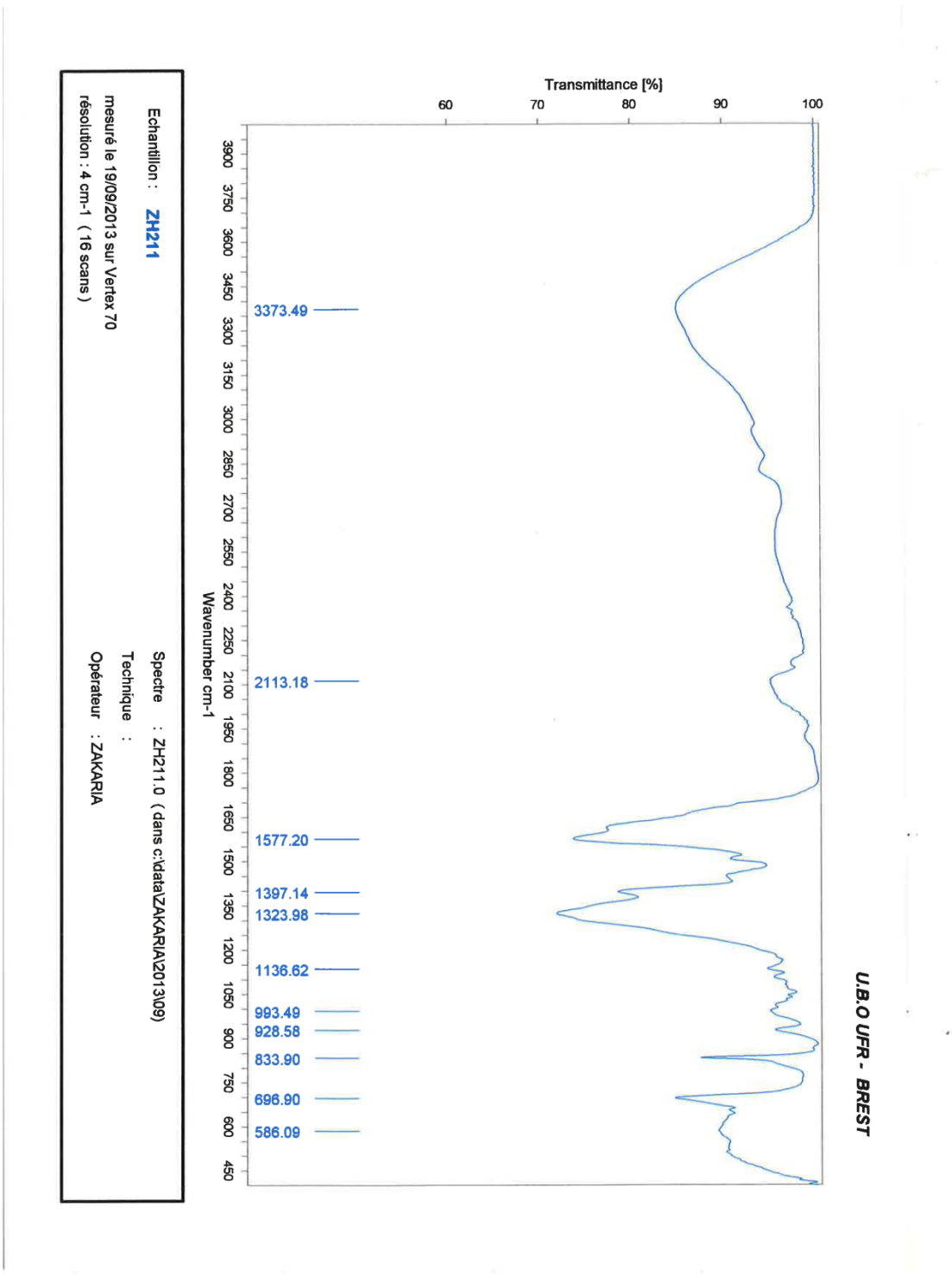


Figure S36. IR spectrum of compound **16**

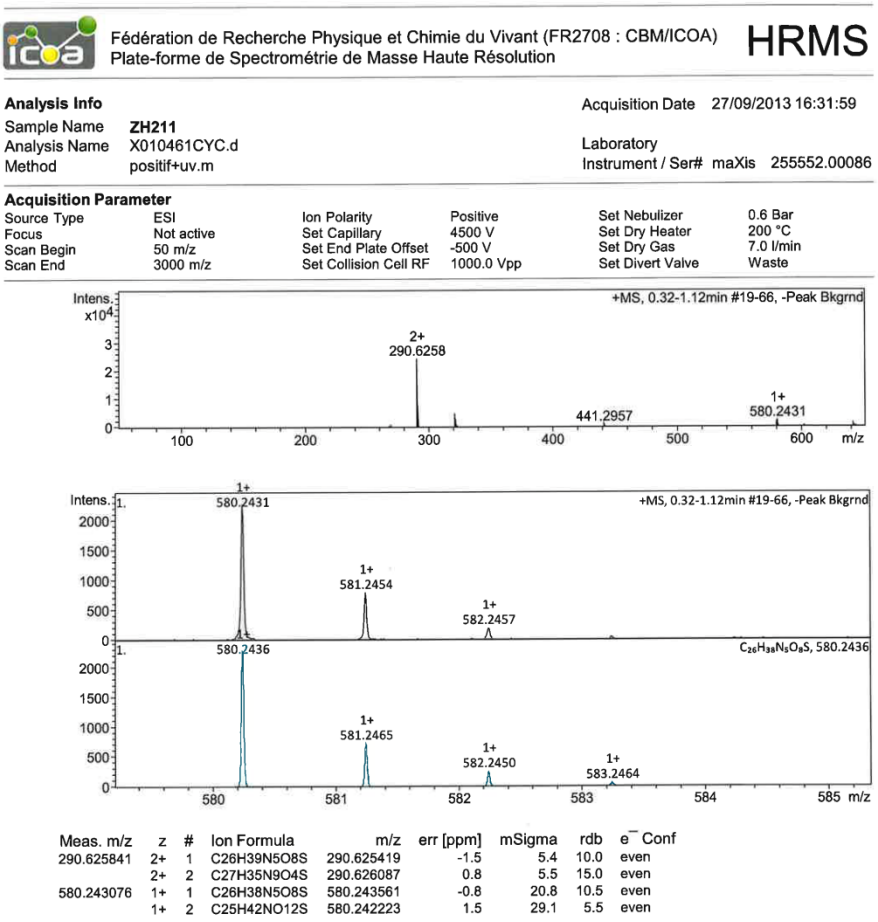
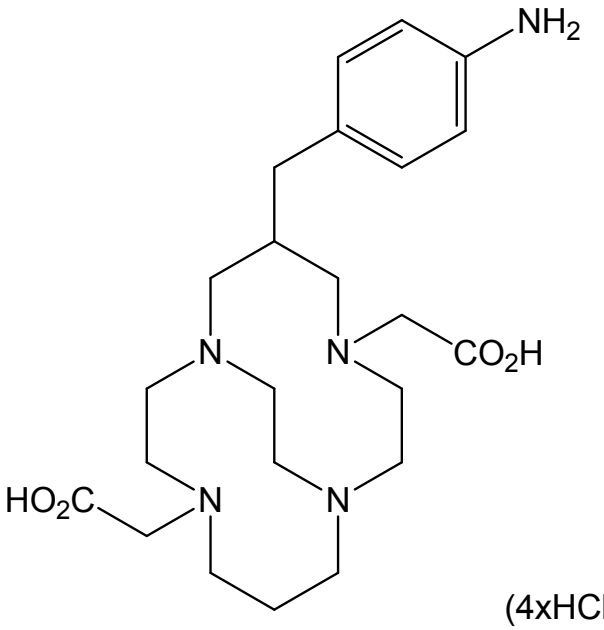


Figure S37. HRMS spectrum (ESI) of compound **16**

Compound 17:



(4xHCl)

Chemical Formula: $C_{23}H_{37}N_5O_4$
Exact Mass: 447,2846
Molecular Weight: 447,5710
m/z: 447.2846 (100.0%), 448.2879 (24.9%), 449.2913 (3.0%), 448.2816 (1.8%)

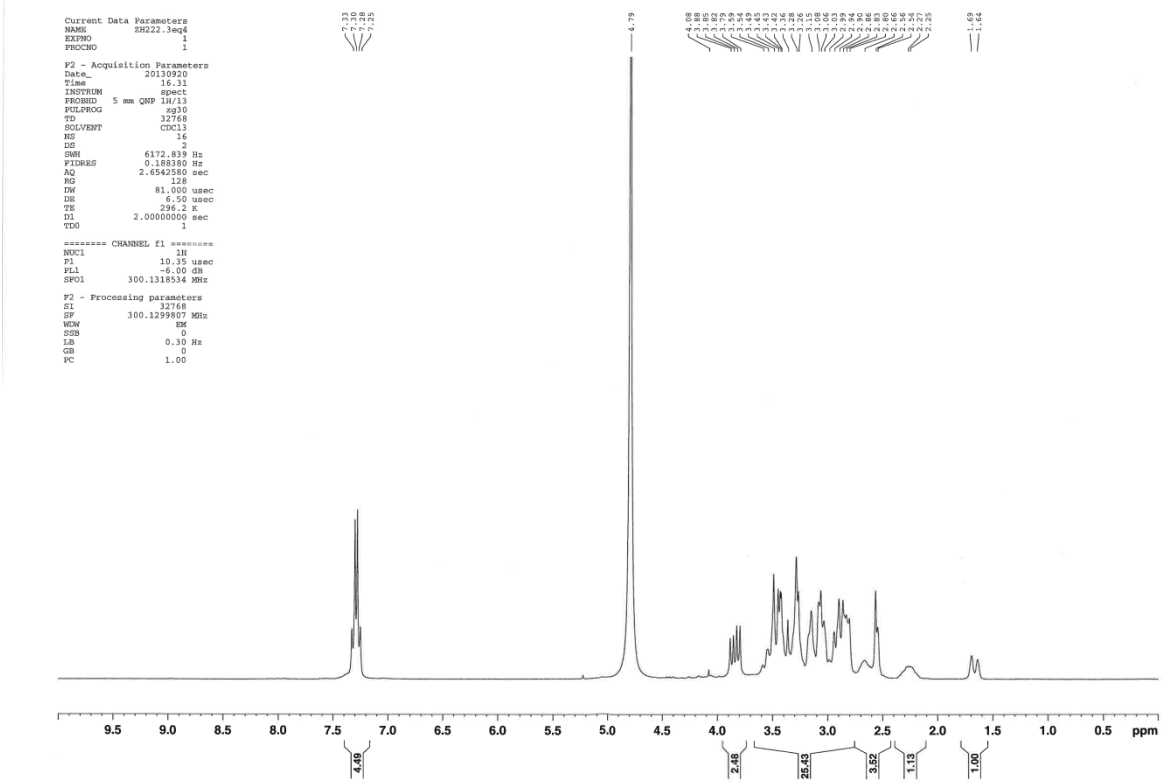


Figure S38. ^{1}H NMR spectrum (300 MHz, D_2O, 25 °C) of compound **17**

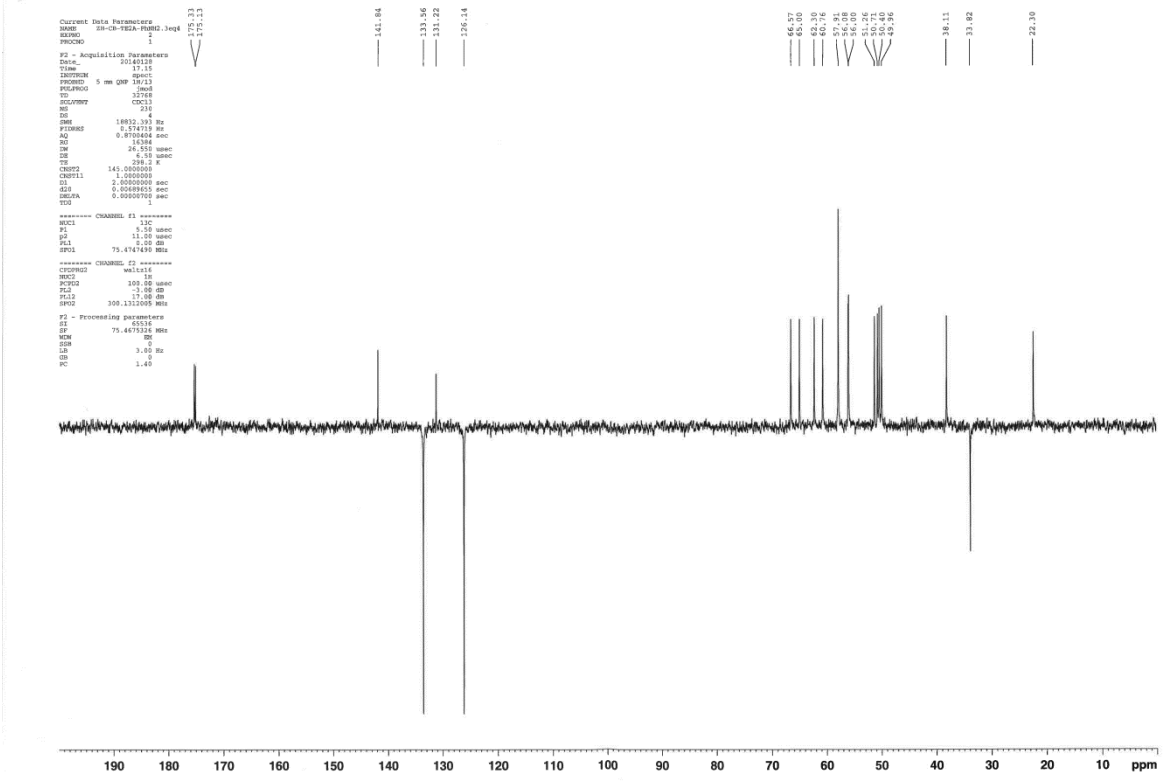


Figure S39. ^{13}C NMR spectrum (300 MHz, D_2O, 25 °C) of compound **17**

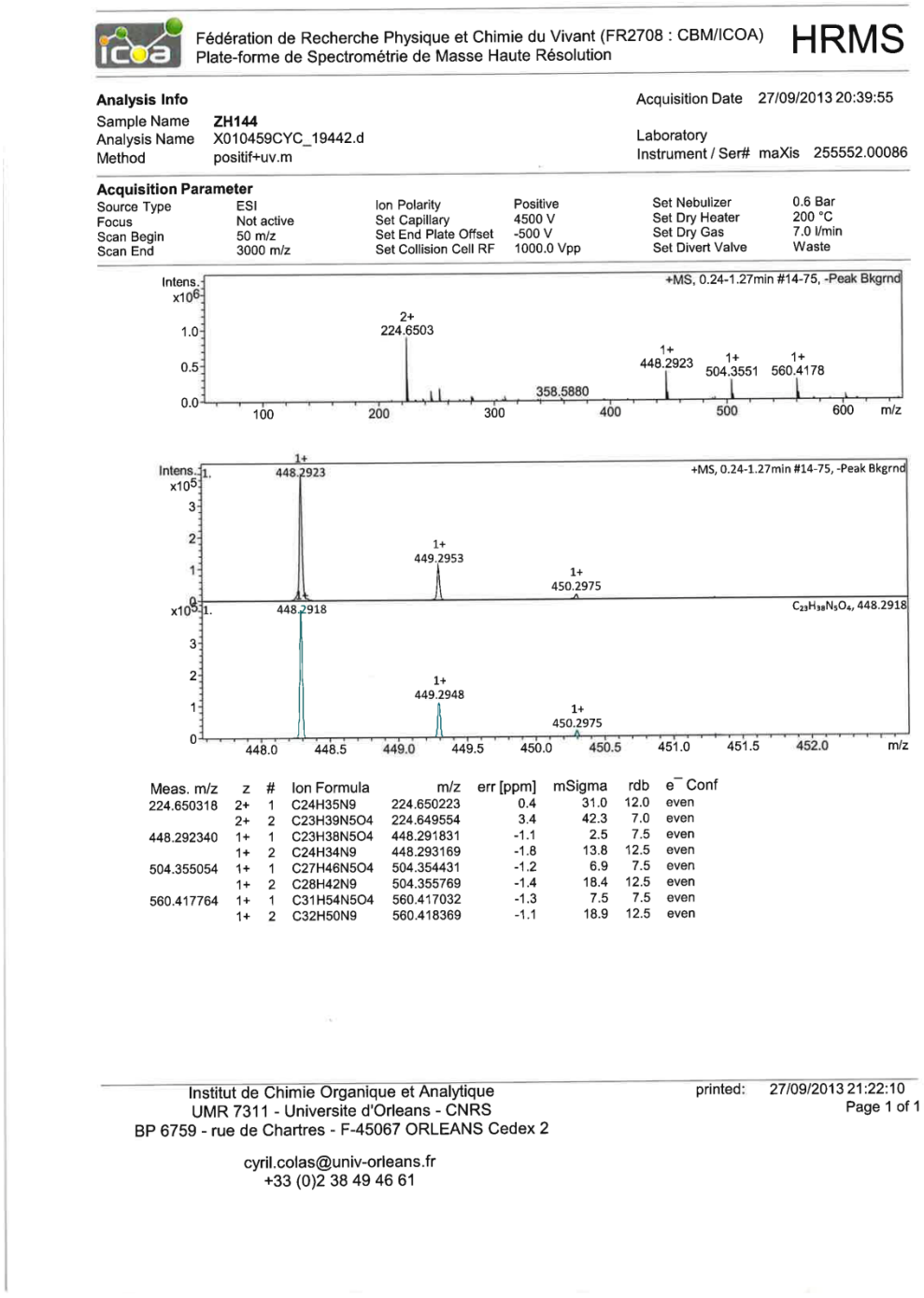
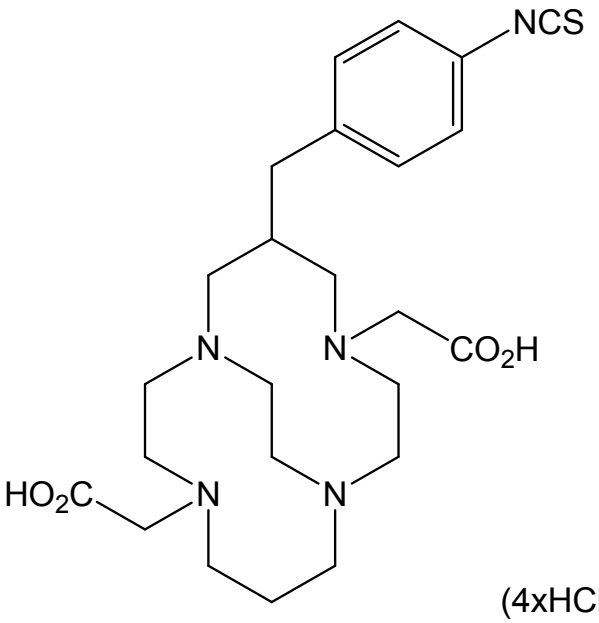


Figure S40. HRMS spectrum (ESI) of compound **17**

Compound 18:



(4xHCl)

Chemical Formula: $C_{24}H_{35}N_5O_4S$
Exact Mass: 489,2410
Molecular Weight: 489,6308
m/z: 489.2410 (100.0%), 490.2443 (26.0%), 491.2368 (4.5%), 491.2477 (3.2%), 490.2380 (1.8%), 492.2401 (1.2%)

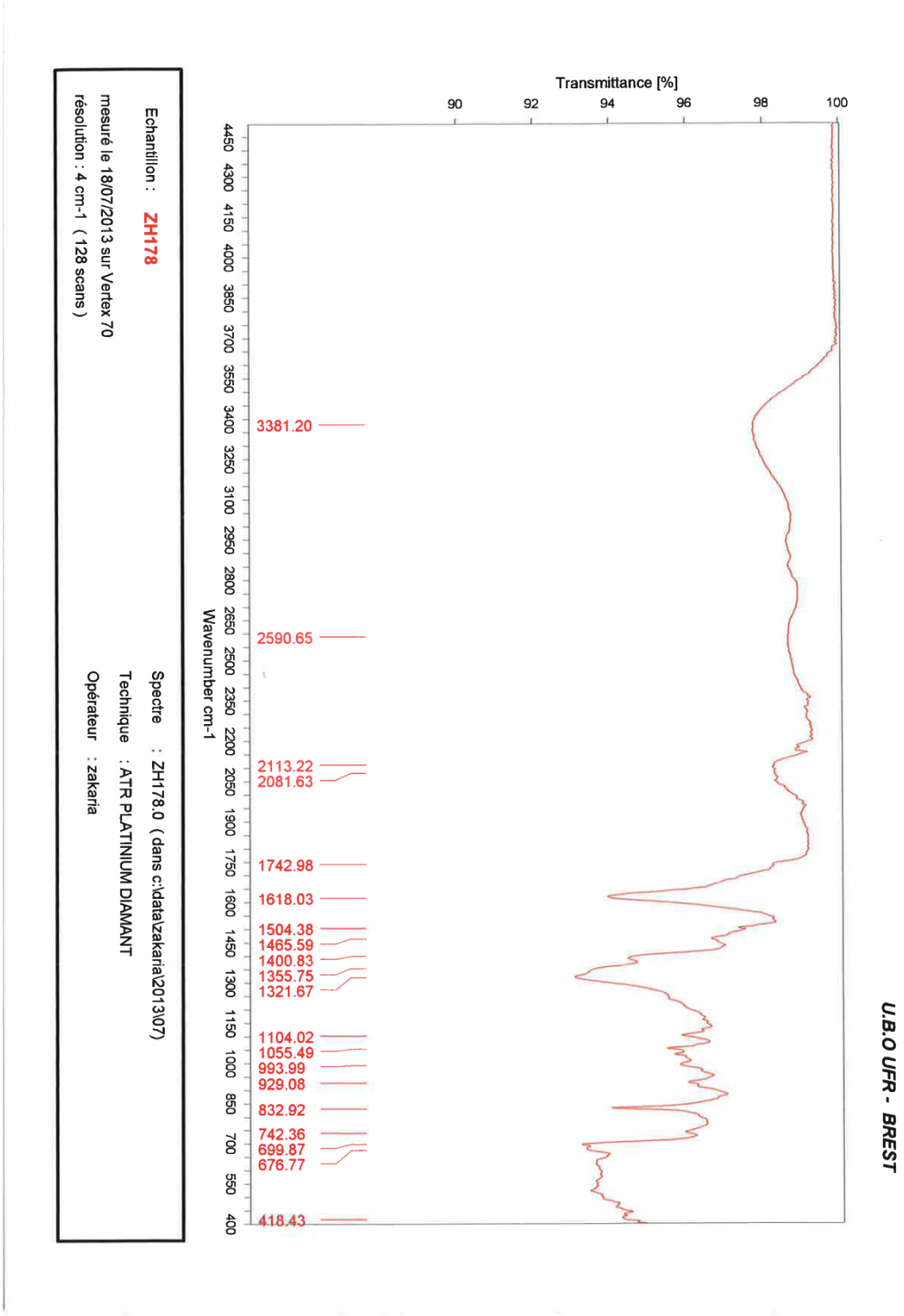
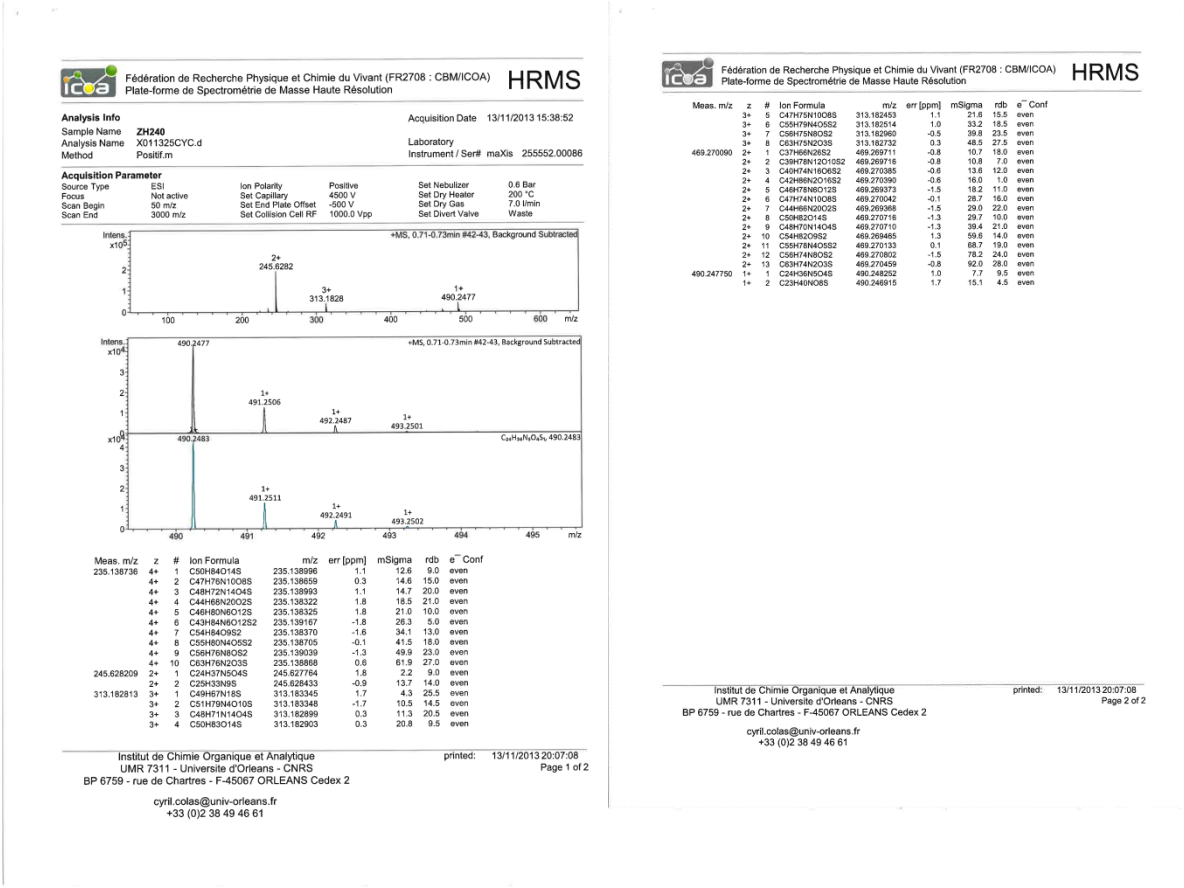


Figure S41. IR spectrum of compound **18**



Fédération de Recherche Physique et Chimie du Vivant (FR2708 : CBM/ICOA)
Plate-forme de Spectrométrie de Masse Haute Résolution

HRMS

Analysis Info

Sample Name	**ZH240**	Acquisition Date	13/11/2013 15:38:52
Analysis Name	X011325CYC.d	Laboratory	
Method	Positif.m	Instrument / Ser#	maXis 255552.00086

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	7.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	1000.0 Vpp	Set Divert Valve	Waste

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
235.138736	4+	1	C50H84O14S	235.138996	1.1	12.6	9.0	even
	4+	2	C47H76N10O8S	235.138659	0.3	14.6	15.0	even
	4+	3	C48H72N14O4S	235.138993	1.1	14.7	20.0	even
	4+	4	C44H68N20O2S	235.138322	1.8	18.5	21.0	even
	4+	5	C46H80N6O12S	235.138325	1.8	21.0	10.0	even
	4+	6	C43H84N6O12S2	235.139167	-1.8	28.3	5.0	even
	4+	7	C54H84O9S2	235.138370	-1.6	34.1	13.0	even
	4+	8	C55H80N4O5S2	235.138705	-0.1	41.5	18.0	even
	4+	9	C56H76N8OS2	235.139039	-1.3	49.9	23.0	even
	4+	10	C63H76N2O3S	235.138868	0.6	61.9	27.0	even
245.628209	2+	1	C24H37N5O4S	245.627764	1.8	2.2	9.0	even
	2+	2	C25H33N9S	245.628433	-0.9	13.7	14.0	even
313.182813	3+	1	C49H67N18S	313.183345	1.7	4.3	25.5	even
	3+	2	C51H79N4O10S	313.183348	-1.7	10.5	14.5	even
	3+	3	C48H71N14O4S	313.182899	0.3	11.3	20.5	even
	3+	4	C50H83O14S	313.182903	0.3	20.8	9.5	even

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Fédération de Recherche Physique et Chimie du Vivant (FR2708 : CBM/ICOA)
Plate-forme de Spectrométrie de Masse Haute Résolution

HRMS

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
	3+	5	C47H75N10O8S	313.182453	1.1	21.8	15.5	even
	3+	6	C55H79N4O5S2	313.182514	1.0	33.2	18.5	even
	3+	7	C56H75N8OS2	313.182960	-0.5	39.8	23.5	even
	3+	8	C63H75N2O3S	313.182732	0.3	48.5	27.5	even
469.270090	2+	1	C37H66N26S2	469.269711	-0.8	10.7	18.0	even
	2+	2	C39H78N12O10S2	469.269716	-0.8	10.8	7.0	even
	2+	3	C40H74N18O6S2	469.270385	-0.6	13.6	12.0	even
	2+	4	C42H86N2O16S2	469.270390	-0.6	16.0	1.0	even
	2+	5	C46H78N8O12S	469.269373	-1.5	18.2	11.0	even
	2+	6	C47H74N10O8S	469.270042	-0.1	28.7	16.0	even
	2+	7	C44H66N20O2S	469.269368	-1.5	29.0	22.0	even
	2+	8	C50H82O14S	469.270716	-1.3	29.7	10.0	even
	2+	9	C48H70N14O4S	469.270710	-1.3	39.4	21.0	even
	2+	10	C54H82O9S2	469.269465	1.3	59.6	14.0	even
	2+	11	C55H78N4O5S2	469.270133	0.1	68.7	19.0	even
	2+	12	C56H74N8OS2	469.270802	-1.5	78.2	24.0	even
	2+	13	C63H74N2O3S	469.270459	-0.8	92.0	28.0	even
490.247750	1+	1	C24H36N5O4S	490.248252	1.0	7.7	9.5	even
	1+	2	C23H40NO6S	490.248915	1.7	15.1	4.5	even

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Figure S42. HRMS spectrum (ESI) of compound **18**

Table TS1. Conditions used to optimize the ^{64}Cu radiolabeling of 9E7.4-CSN-Ph-te2a and ^{64}Cu-te2a-Ph-NCS

Test n°	Molar ratio : Immunoconjugate/Cu or Ligand/Cu	Acetate buffer (pH)	Time (min)	Temp.
1	1.1	7	5, 15, 30, 60	RT
2	1.1	7	5, 15, 30, 60	40°C
3	1.1	5	5, 15, 30, 60	40°C
4	1.1	7	30	4°C

Table TS2. In vitro competition assay of ^{64}Cu-9E7.4-CSN-Ph-te2a and ^{64}Cu-te2a-Ph-NCS against EDTA (10000 eq.) (conditions are presented in experimental part).

	^{64}Cu-9E7.4-CSN-Ph-te2a	^{64}Cu-te2a-Ph-NCS
Before incubation of EDTA	93 %	98 %
After one night incubation at RT with EDTA	51 %	56 %

Results are expressed as radiolabeling yield

Table TS3. Raw quantitative data extracted from PET-CT image of subcutaneous multiple myeloma tumor bearing mice at 2 h and 20 h post-injection (PI) of ^{64}Cu-9E7.4-CSN-Ph-te2a.

2 h Post Injection							
VOI	**Tumor**	**Liver**	**Heart**	**Muscle (back leg)**	**bladder**	**gut**	**kidney**
Max (kBq/ml)	364.6	1625.9	1198.2	84.5	220.8	1351.6	542
Average	178.4	1136.5	300.4	41.1	116.4	522.7	217.4
Standard	95.9	244.5	223.9	14.8	40.9	212.9	121.9
Volume (mm3)	343	343	343	38.9	53.7	340	13
20 h Post Injection							
VOI	**Tumor**	**Liver**	**Heart**	**Muscle (back leg)**	**bladder**	**gut**	**kidney**
Max (kBq/ml)	279.9	505.5	221.4	41.8	146.2	361.4	164,1
Average	112.7	315.9	110.2	17.0	68	164.5	96
Standard	60.2	85.8	39.4	6.4	27.6	68.7	37.9
Volume (mm3)	339	339	339	40.3	53.9	339	13

The volumes of interest (VOI) were manually plotted to assess the uptake of organs and tumor (in kBq/ml) by using AW Volume Share 5 (AW 4.6) software from General Electric.

Table TS4. Radiolabeling monitoring of ^{64}Cu-9E7.4-CSN-Ph-te2a (a) and ^{64}Cu-te2a-Ph-NCS (b) at different pH and temperature conditions.

	pH 7; RT		**pH 7; 40°C**		**pH 5; 40°C**		**pH 7; 4°C**	
	a	b	a	b	a	b	a	b
5 min	62 %	30 %	63 %	72 %	73 %	64 %	-	-
15 min	78 %	74 %	86 %	84 %	86 %	70 %	-	-
30 min	80 %	-	89 %	-	86 %	-	71 %	-
60 min	82 %	88 %	90 %	90 %	91 %	93 %	-	-

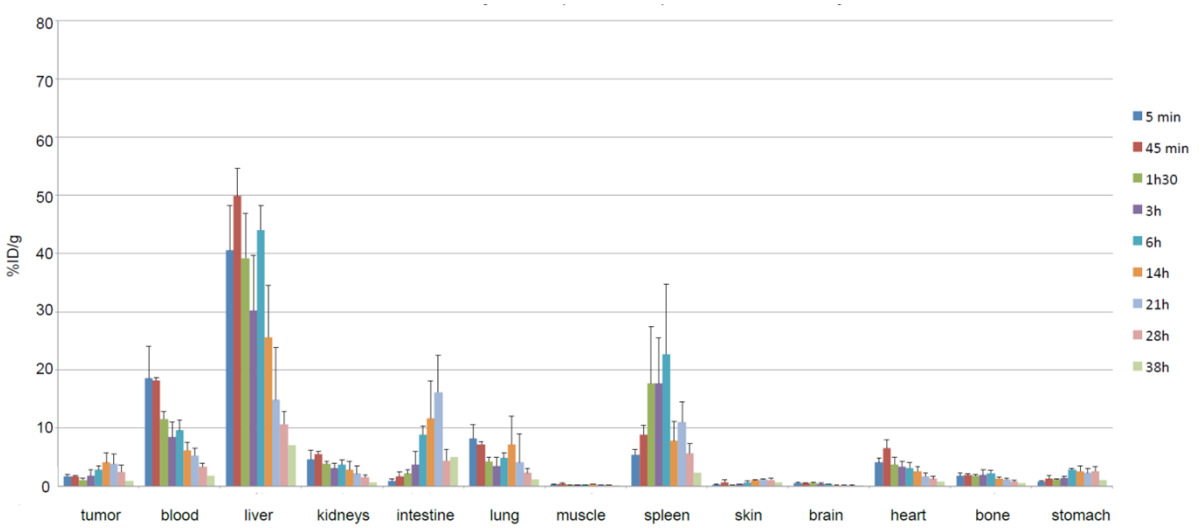

Figure S43. Biodistribution of ^{125}I-9E7.4 in subcutaneous multiple myeloma tumor bearing mice showing the large uptake in liver and gut especially at 21h post-injection.

Figure S44. Characterization of 9E7.4 mAb specificity by flow cytometry. Staining with 9E7.4 mAb (black histogram) and an IgG2a,κ isotype control (white histogram) followed by a PE-conjugated anti-rat IgG secondary antibody was performed on CD138+ 5T33 cell line. Flow cytometry was performed on a BD FACS Calibur™ Flow Cytometry System.