Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supporting Information for

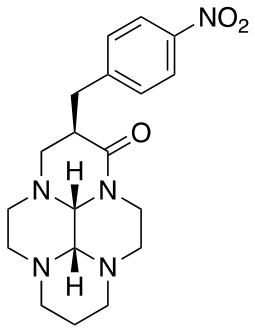
Synthesis of a *C*-functionalized TE1PA and comparison with analogues. Example of bioconjugation on 9E7.4 mAb for multiple myeloma ^{64}Cu-PET imaging

Thomas Le Bihan,[a] Anne-Sophie Navarro,[b] Nathalie Le Bris,[a] Patricia Le Saëc,[b] Sébastien Gouard,[b] Ferid Haddad,[c] Jean-François Gestin,[b] Michel Chérel,[b,d] Alain Faivre-Chauvet,[b,*] Raphaël Tripier[a,*]

[a.] *Université de Brest, UMR-CNRS 6521 / IBSAM, UFR Sciences et Techniques, 6 Avenue Victor le Gorgeu, C.S. 93837, 29238 Brest, France.*
E-mail : raphael.tripier@univ-brest.fr
[b.] *Université de Nantes, Centre de Recherche en Cancérologie et Immunologie Nantes Angers (CRCINA), Unité INSERM 892 – CNRS 6299, 8 quai Moncousu, BP 70721 44007 Nantes Cedex, France.*
[c.] *Groupement d'Intérêt Public ARRONAX, 44800 Saint-Herblain, France*
[d.] *Institut de Cancérologie de l'Ouest, 44800 Saint-Herblain, France.*

Table of content

Coumpound 1



Formula: $C_{19}H_{25}N_5O_3$

Exact Mass: *371.196 g.mol^{-1}*

Molecular Weight: *371.441 g.mol^{-1}*

Description: *white powder*

^{1}H NMR (300 MHz, $CDCl_3$, 25°C) δ 8.07 (d, *J* = 8.7 Hz, 2H, C*H*$_{Ar}$ $PhNO_2$), 7.31 (d, *J* = 8.7 Hz, 2H, C*H*$_{Ar}$ $PhNO_2$), 4.47-4.35 (m, 1H), 4.22 (d, *J* = 3.1 Hz, 1H), 3.47 (dd, *J* = 14.0, 3.8 Hz, 1H, N-C*H*-N), 3.38 (td, *J* = 12.0, 3.3 Hz, 1H), 3.10 (d, *J* = 3.1 Hz, 1H, N-C*H*-N), 3.04 (dd, *J* = 11.2, 2.6 Hz, 1H), 3.00-2.82 (m, 6H), 2.82-2.57 (m, 3H), 2.43-2.28 (m, 2H), 2.24-2.04 (m, 3H), 1.30-1.10 (m, 1H, C*H*$_2$-β-N).

^{13}C NMR (75 MHz, $CDCl_3$, 25°C) δ 170.4 (*C*O), [147.8, 146.6] (*C*$_{Ar}$ $PhNO_2$), [129.9, 123.7] (*C*H$_{Ar}$ $PhNO_2$), [76.00, 70.9] (N-*C*H-N), [55.9, 53.9, 53.2, 53.0, 44.4, 43.9, 40.6] (*C*H_2-α-N), 36.7 (*C*H_2-β-N), 35.1 (*C*H_2-$PhNO_2$), 19.6 (*C*H_2-β-N).

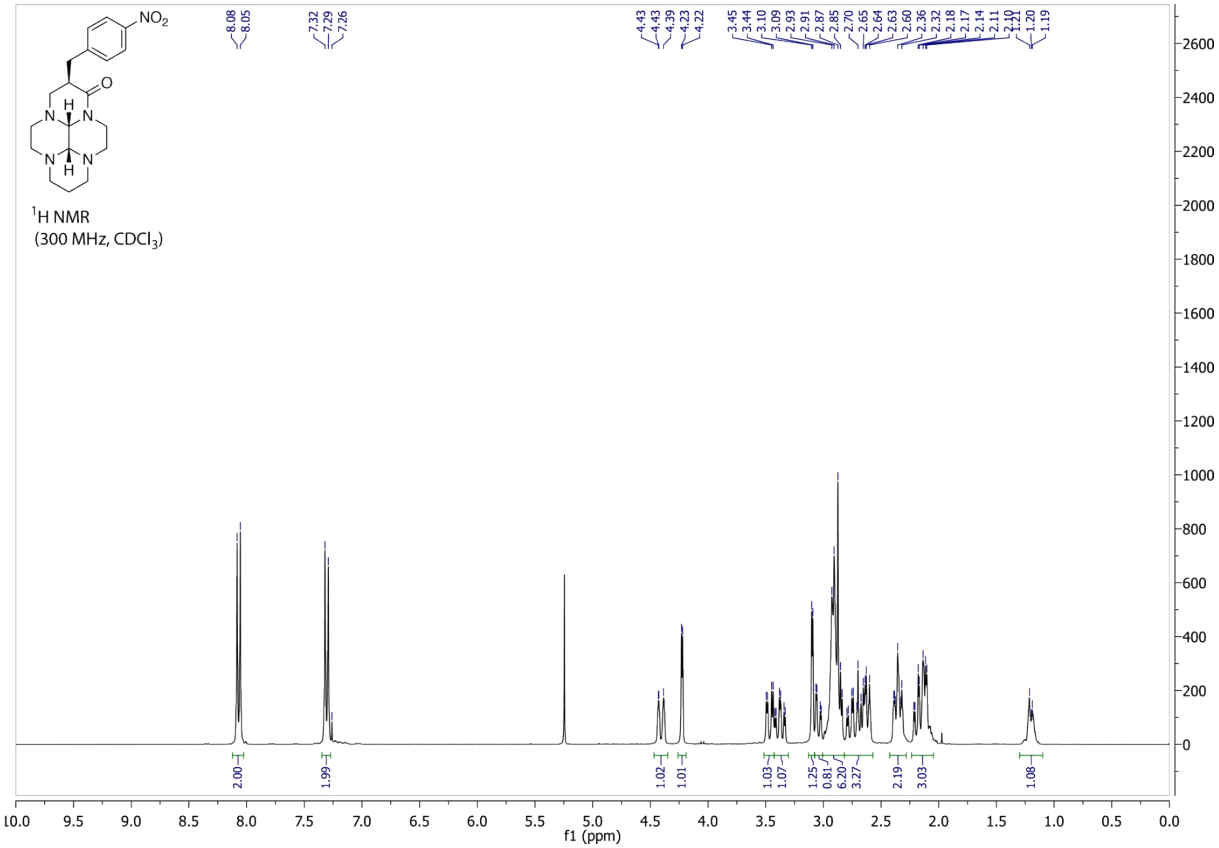


Figure S1: ^{1}H NMR Spectrum (300 MHz, $CDCl_3$, 25°C) of compound **1**

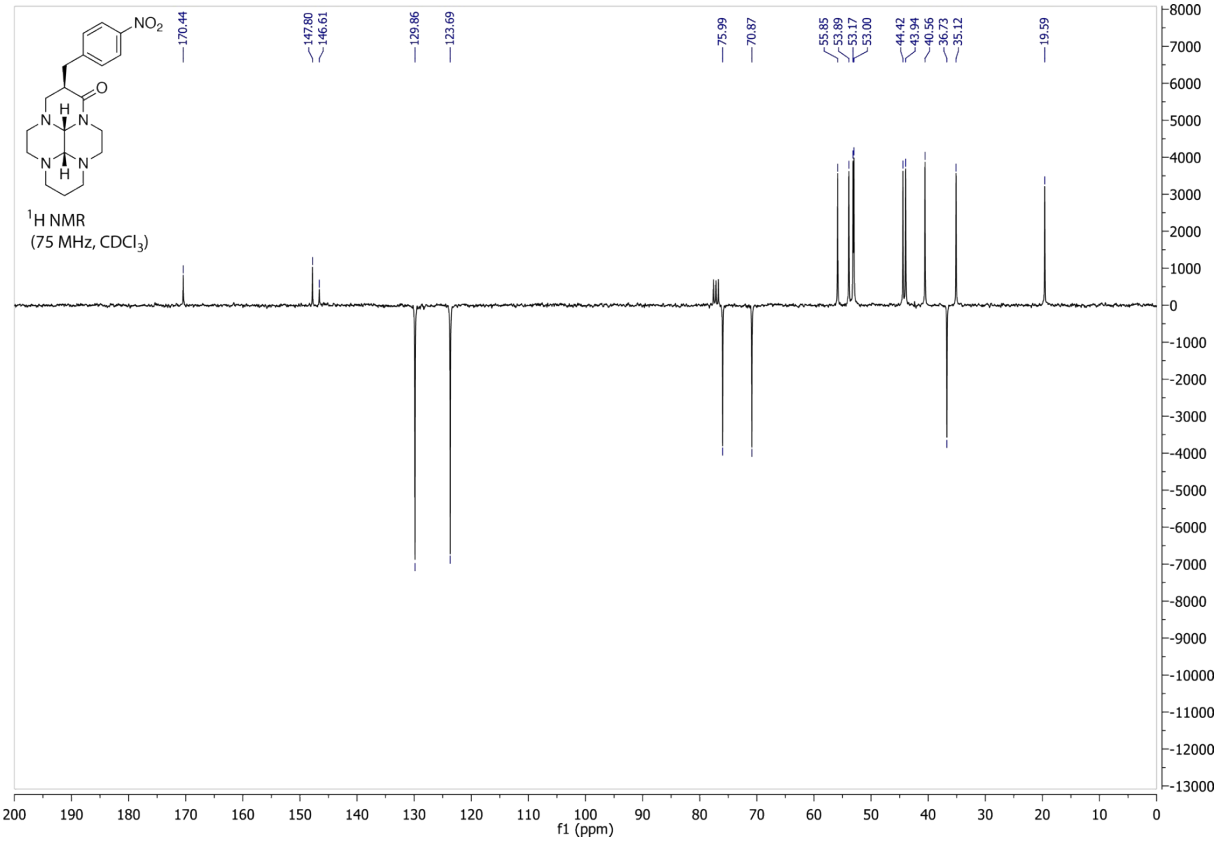
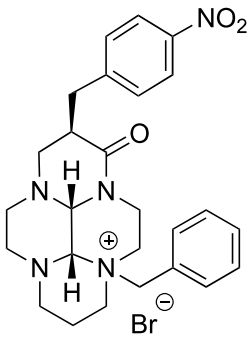


Figure S2 : ^{13}C NMR spectrum (75 MHz, $CDCl_3$, 25°C) of compound **1**



Compound 2

Formula: $C_{26}H_{32}N_5O_3Br$

Exact Mass: *371.196 g.mol^{-1}*

Molecular Weight: *542.478 g.mol^{-1}*

Description: *White Powder*

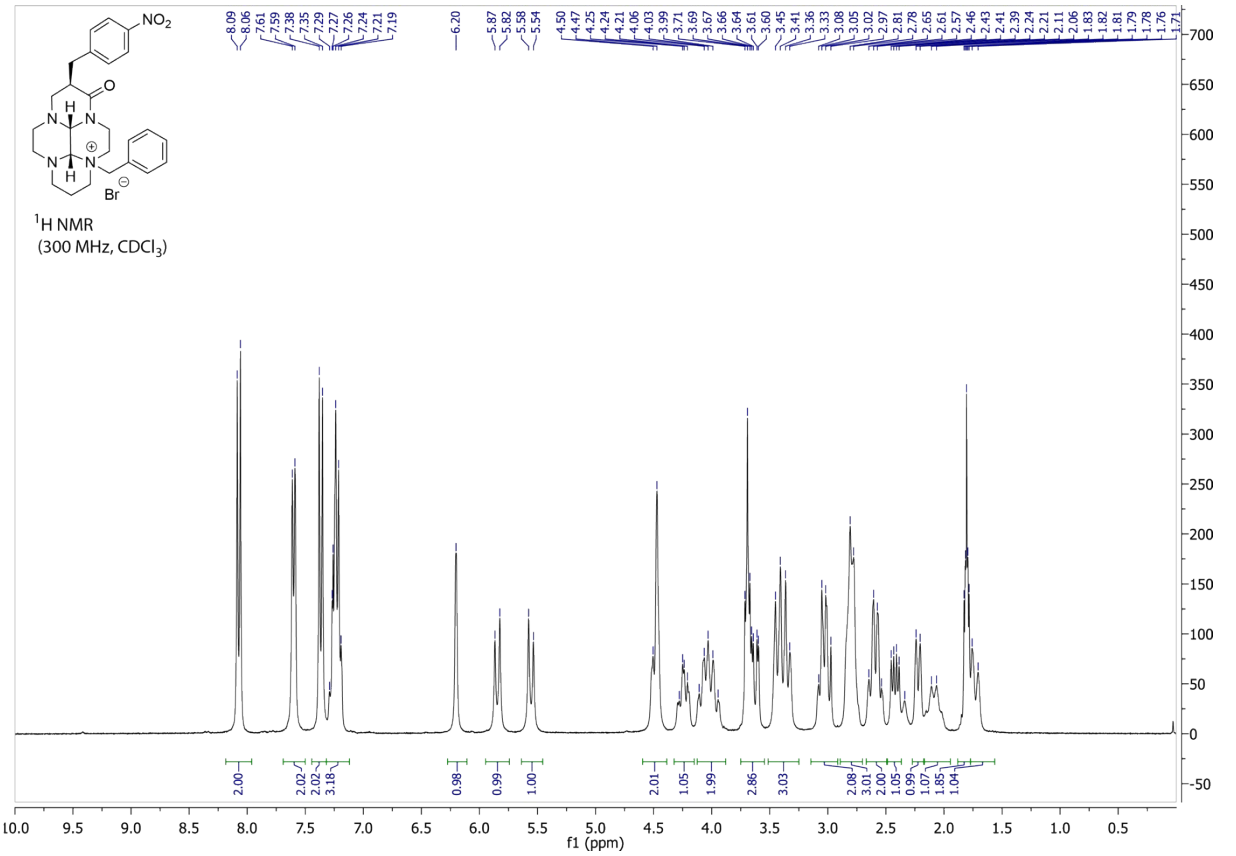


Figure S3: ^{1}H NMR Spectrum (300 MHz, $CDCl_3$, 25°C) of compound **2**

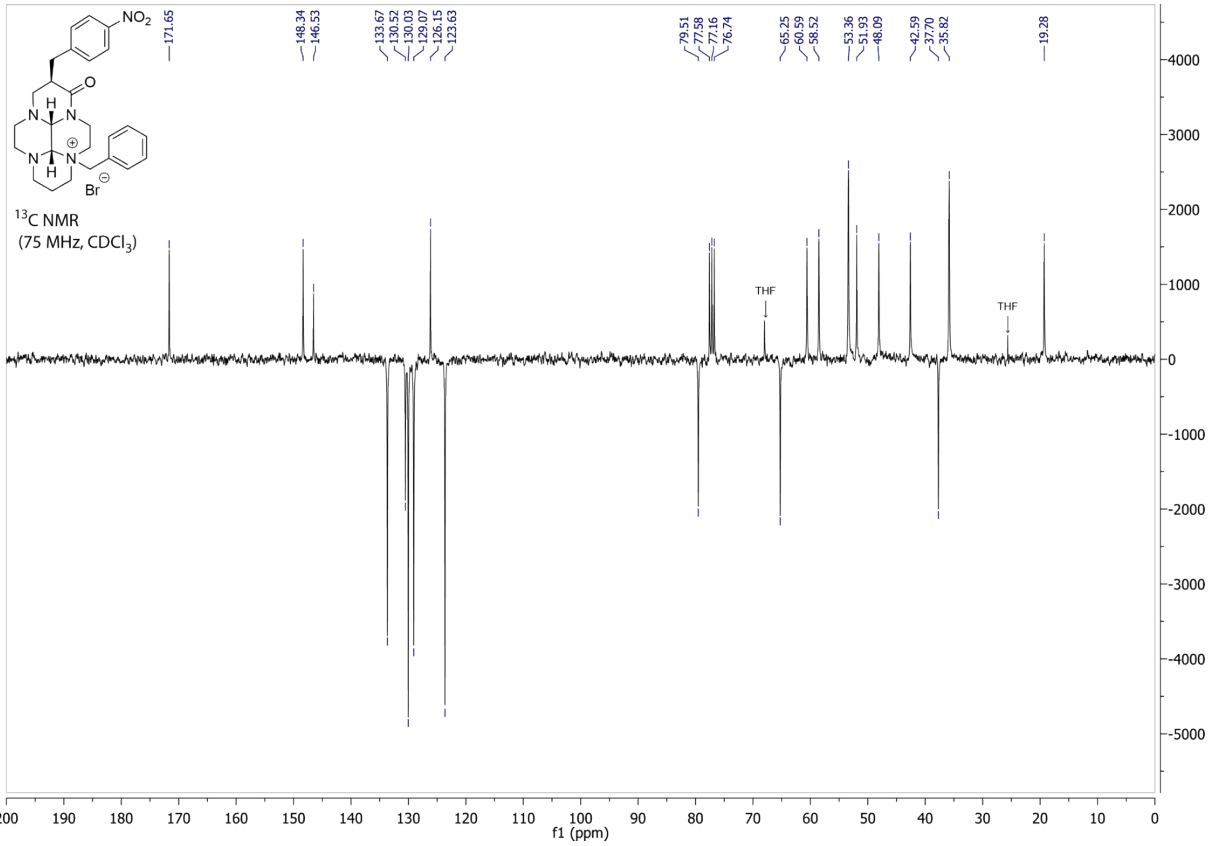


Figure S4: ^{13}C NMR spectrum (75 MHz, $CDCl_3$, 25°C) of compound **2**

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Plate-forme de Spectrométrie de Masse Haute Résolution

HRAM

Analysis Info

Sample Name	**Oxocyclam-gly-N1-benzyl-BnNO2**	Acquisition Date	10/07/2017 14:34:13
Analysis Name	X035623CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
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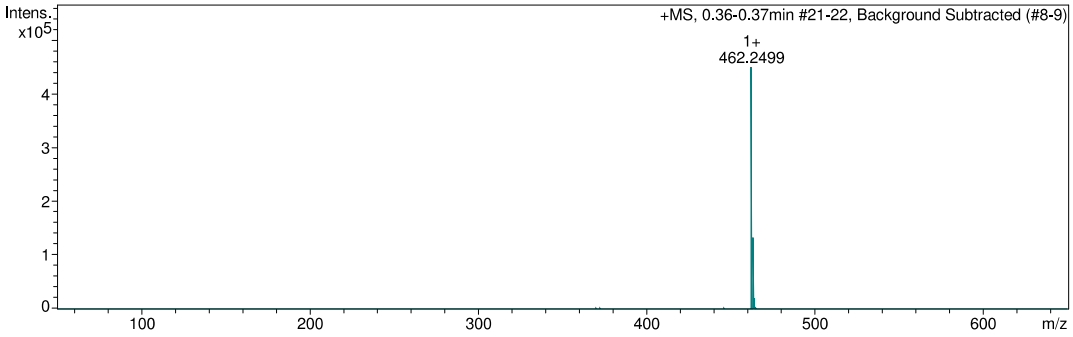
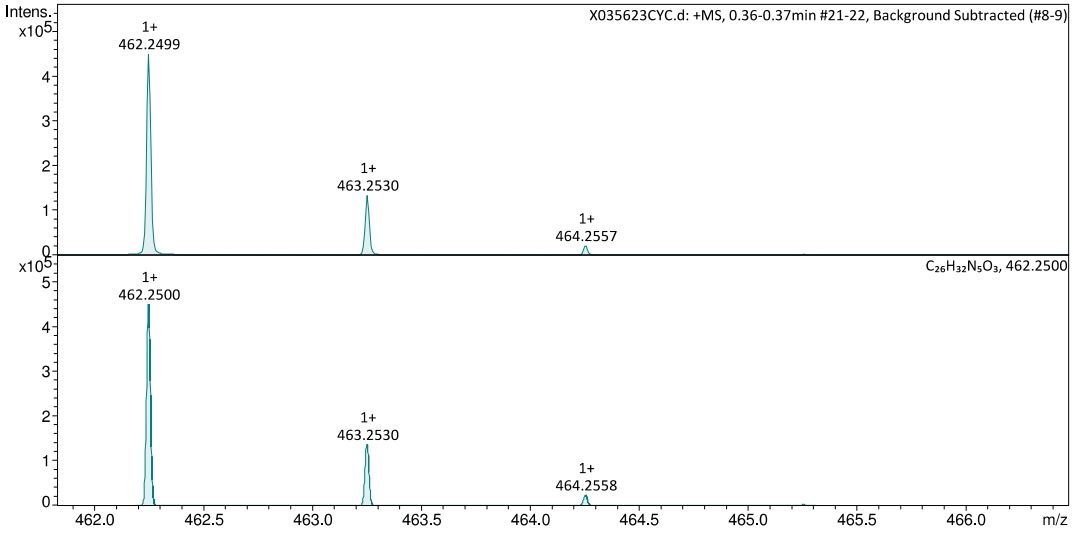
Scan End	2500 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
462.249946	1+	1	C26H32N5O3	462.249966	0.0	4.7	14.0	even

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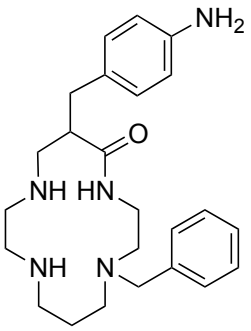
Figure S5: HRMS spectrum (ESI) of compound **2**

Compound 3



Formula: $C_{24}H_{35}N_5O$

Exact Mass: *409.284 g.mol^{-1}*

Molecular Weight: *409.578 g.mol^{-1}*

Description: *yellowish oil*

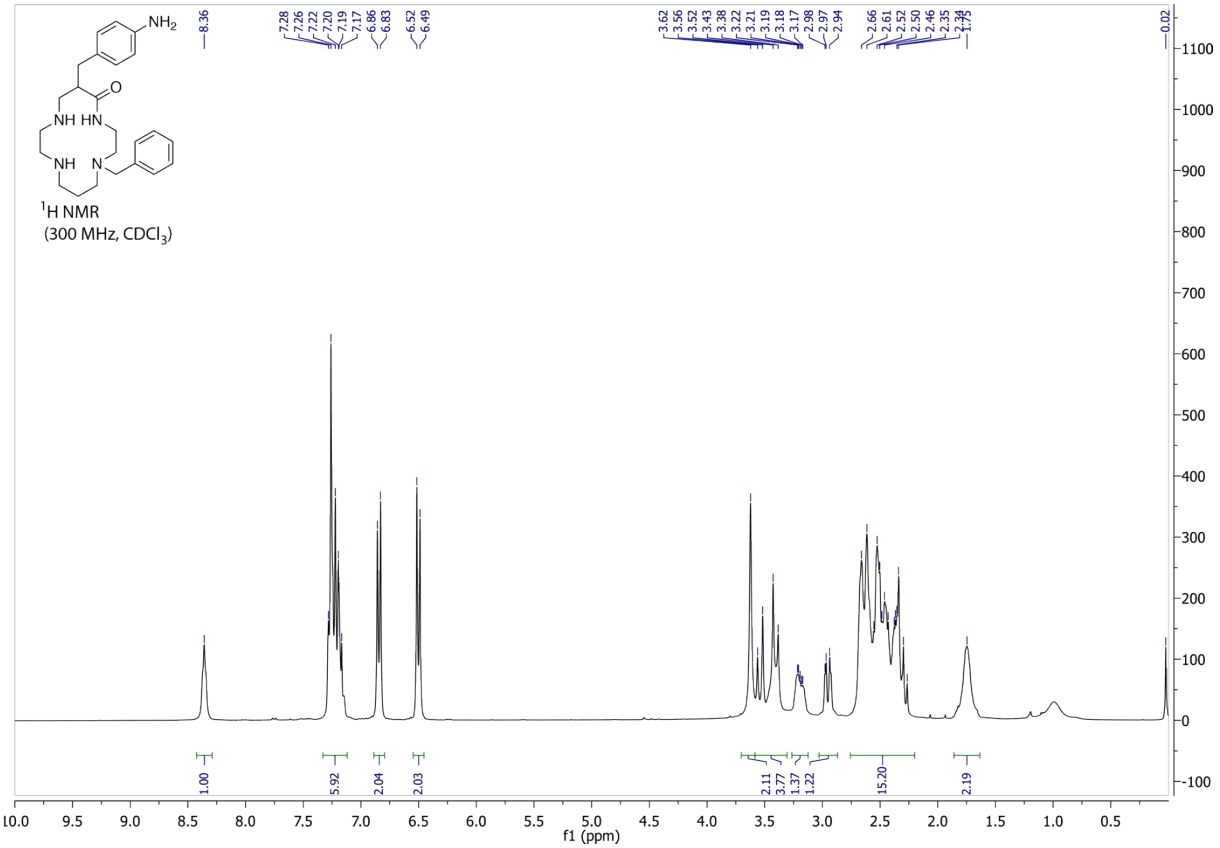


Figure S6: ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 25°C) of compound **3**

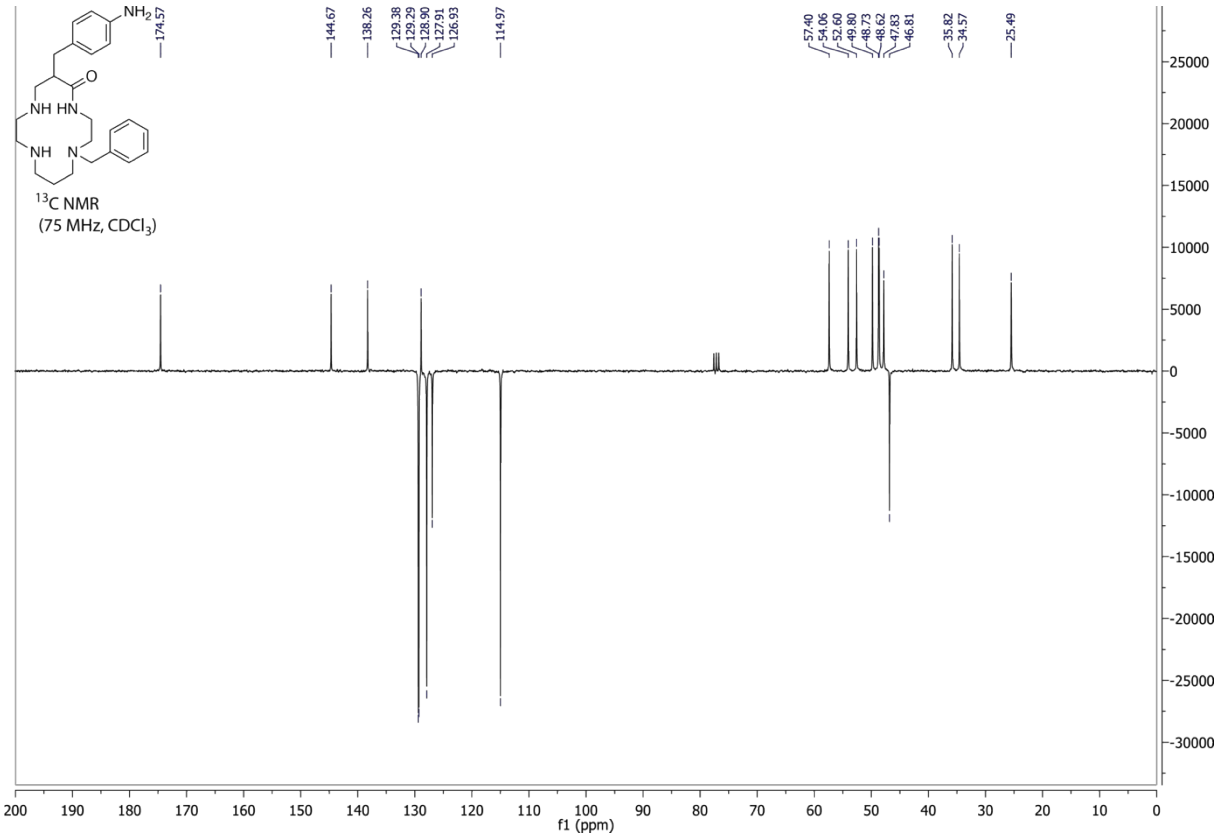


Figure S7: ^{13}C NMR spectrum (75 MHz, $CDCl_3$, 25°C) of compound **3**

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Plate-forme de Spectrométrie de Masse Haute Résolution

HRAM

Analysis Info

Sample Name	**Oxocyclam-N1-benzyl-BnNH2**	Acquisition Date	10/07/2017 14:31:09
Analysis Name	X035621CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
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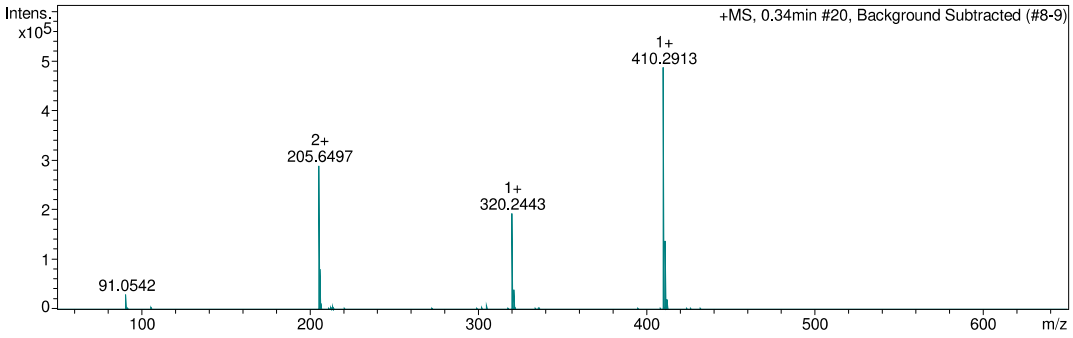
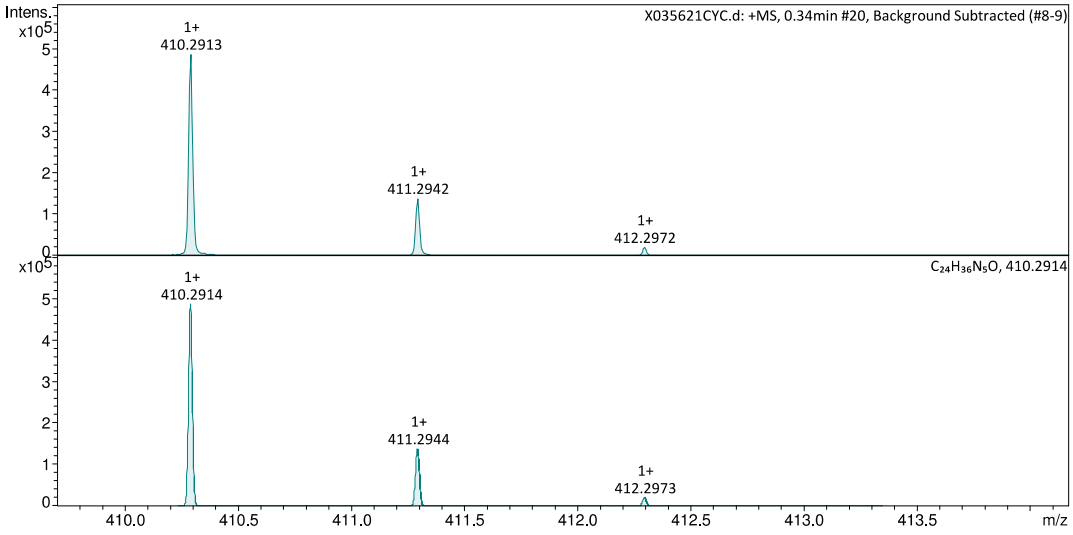
Scan End	2500 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
91.054195	2+	1	C14H14	91.054227	0.3	100.4	9.0	even
205.649673	2+	1	C24H37N5O	205.649357	-1.5	1.3	10.0	even
320.244312	1+	1	C17H30N5O	320.244487	0.5	3.3	6.0	even
410.291304	1+	1	C24H36N5O	410.291437	0.3	0.9	10.0	even

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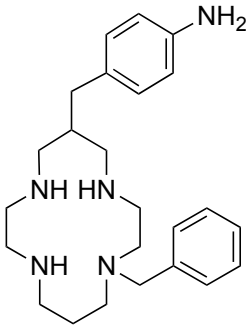
Figure S8: HRMS spectrum (ESI) of compound **3**

Compound 4



Formula: $C_{24}H_{37}N_5$

Exact Mass: *395.305 g.mol^{-1}*

Molecular Weight: *395.595 g.mol^{-1}*

Description: *colorless oil*

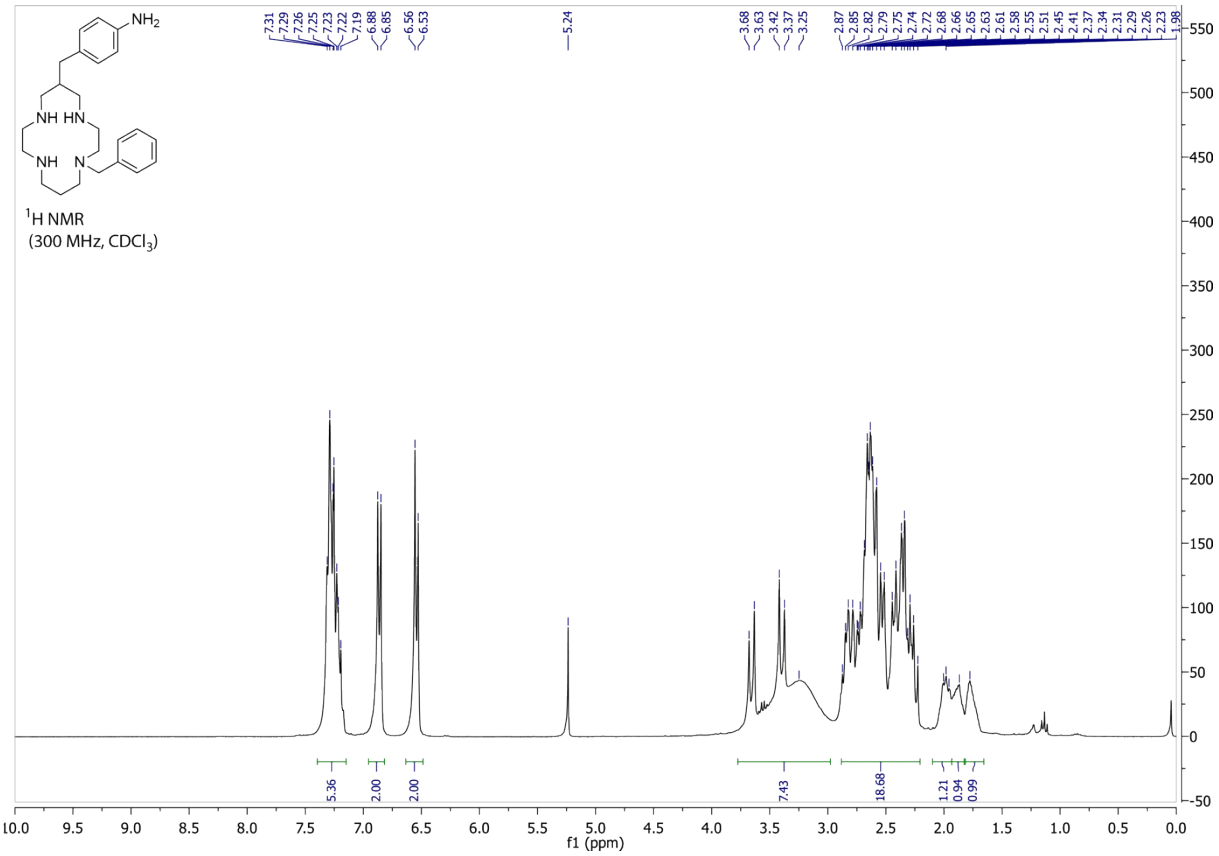


Figure S9:[1]H NMR spectrum (300 MHz, $CDCl_3$, 25°C) of compound **4**

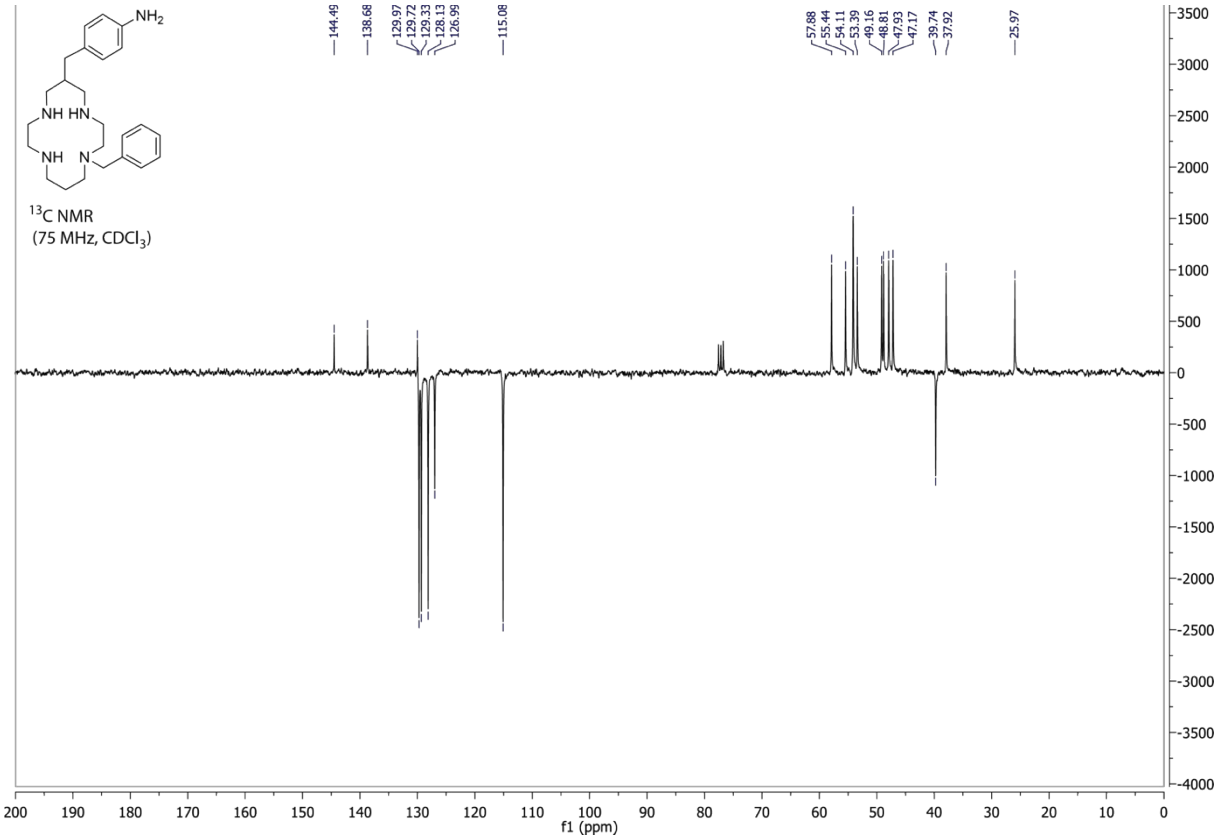


Figure S10: [13]C NMR spectrum (75 MHz, $CDCl_3$, 25°C) of compound **4**

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Plate-forme de Spectrométrie de Masse Haute Résolution

HRAM

Analysis Info

Sample Name	**cyclam-benzyl-PhNH2**	Acquisition Date	11/07/2017 21:32:35
Analysis Name	X035656CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
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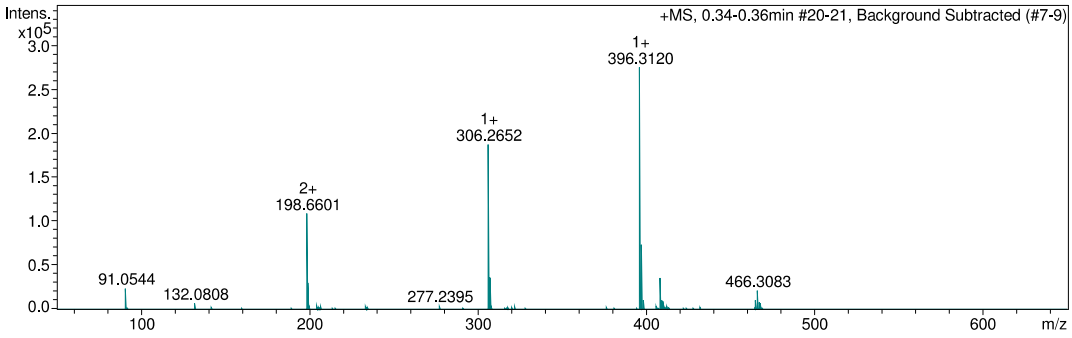
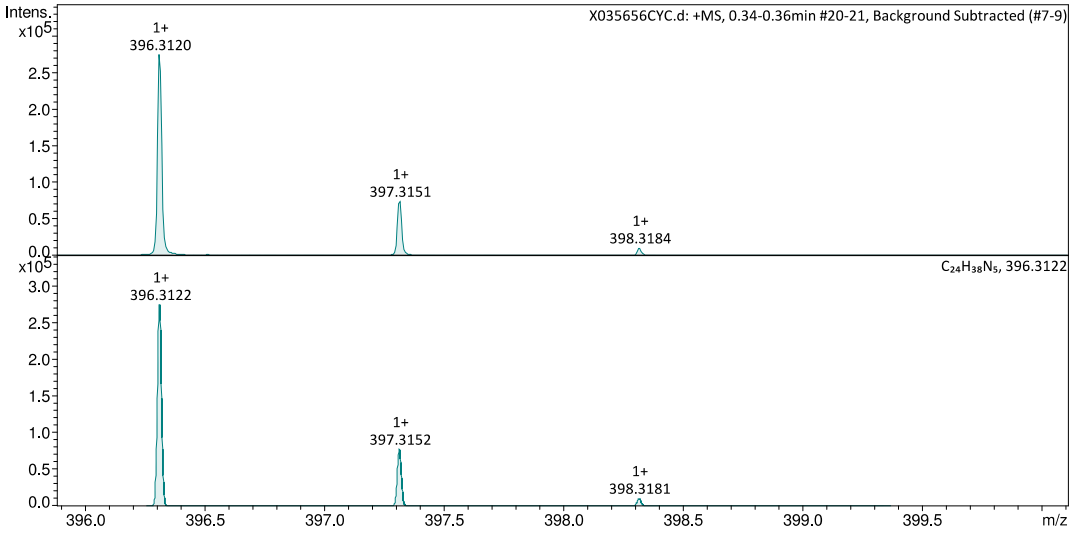
Scan End	2500 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
91.054432	1+	1	C7H7	91.054227	-2.3	8.5	5.0	even
198.660147	2+	1	C24H39N5	198.659725	-2.1	1.8	9.0	even
306.265189	1+	1	C17H32N5	306.265222	0.1	7.6	5.0	even
396.311986	1+	1	C24H38N5	396.312173	0.5	7.6	9.0	even
408.312162	1+	1	C25H38N5	408.312173	0.0	119.1	10.0	even

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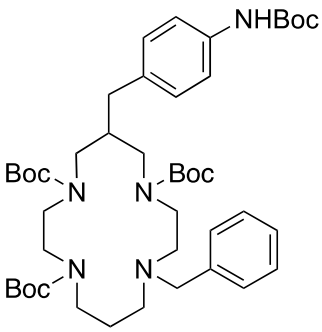
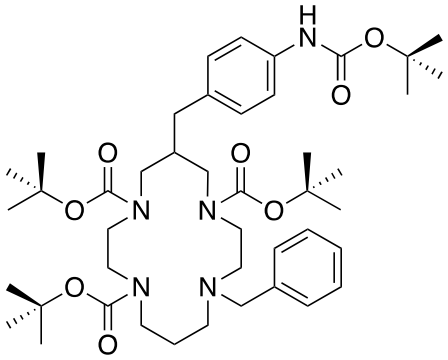
Figure S11: HRMS spectrum (ESI) of compound **4**

Compound 5





Formula: $C_{44}H_{69}N_5O_8$

Exact Mass: *795.515 g.mol*$^{-1}$

Molecular Weight: *796.063 g.mol*$^{-1}$

Description: *yellowish oil*

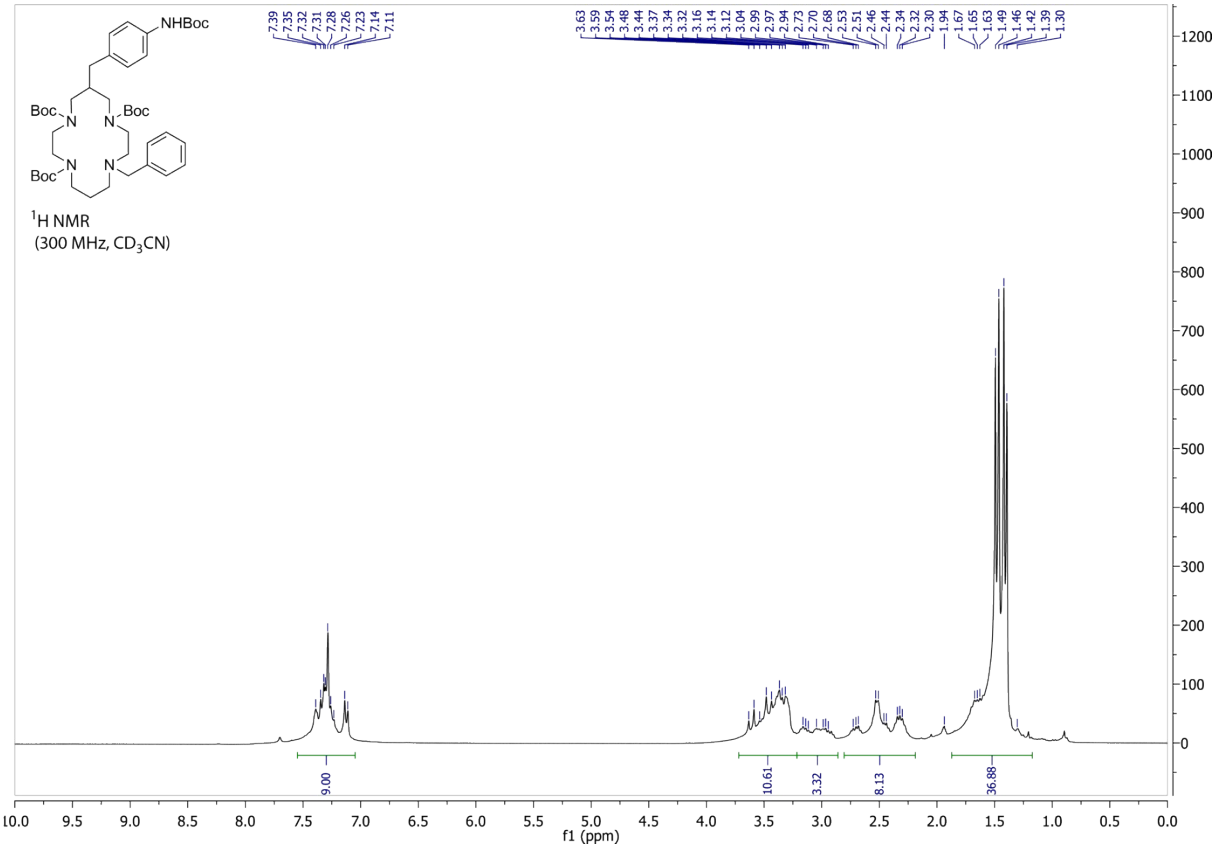


Figure S12: ^{1}H NMR spectrum (300 MHz, CD_3CN, 70°C) of compound **5**

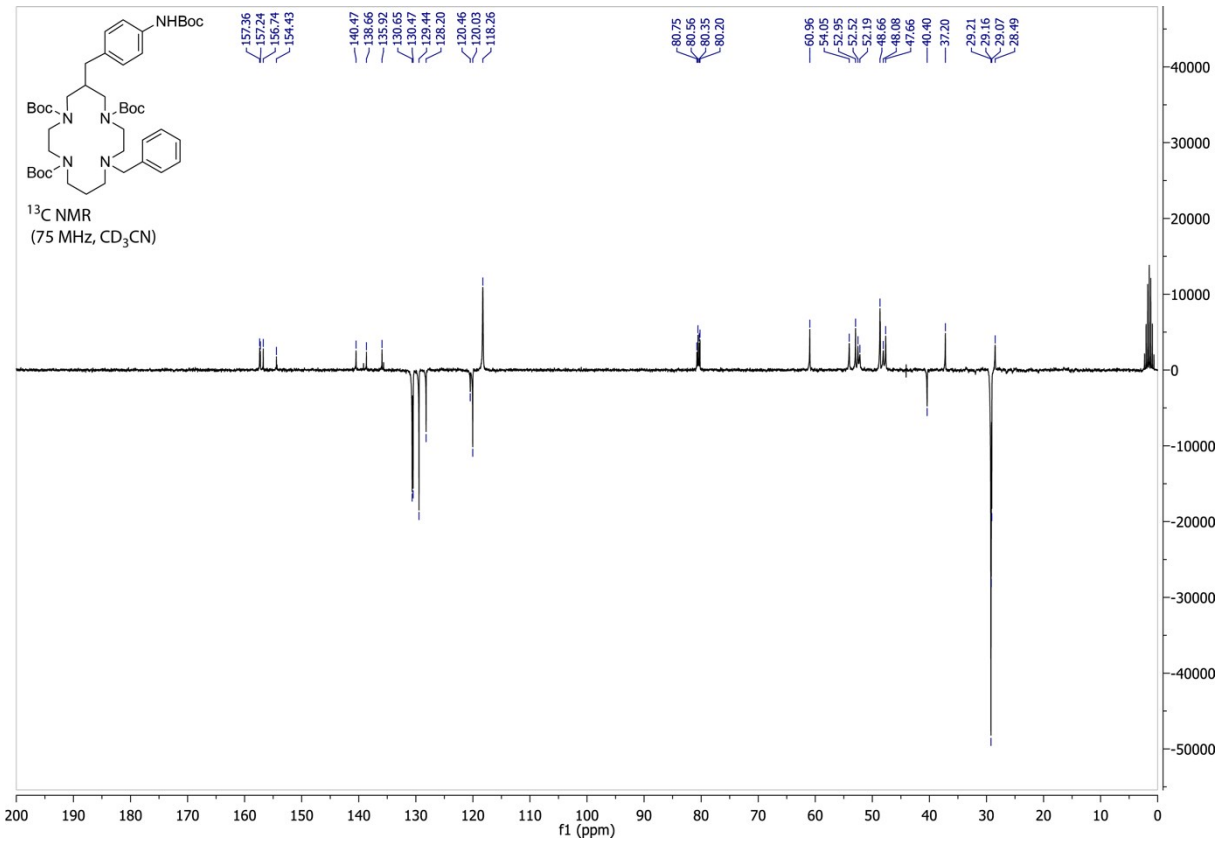


Figure S13: ^{13}C NMR spectrum (75 MHz, CD_3CN, 70°C) of compound **5**

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Plate-forme de Spectrométrie de Masse Haute Résolution

HRMS

Analysis Info

Sample Name	**TLB 64**	Acquisition Date	10/02/2016 13:16:15
Analysis Name	X023259CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
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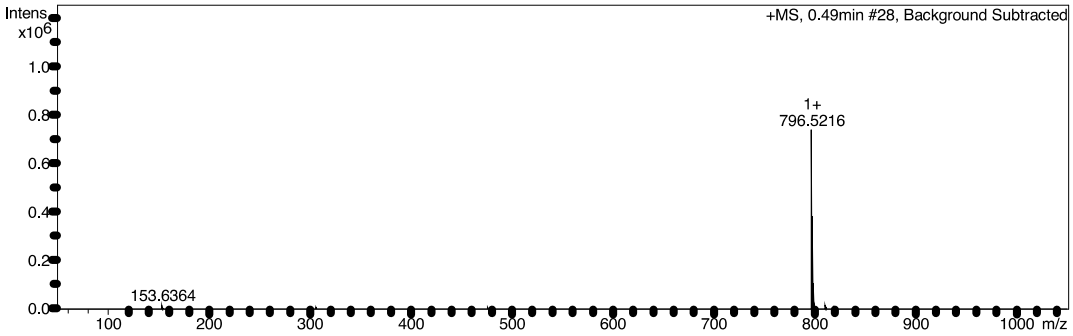
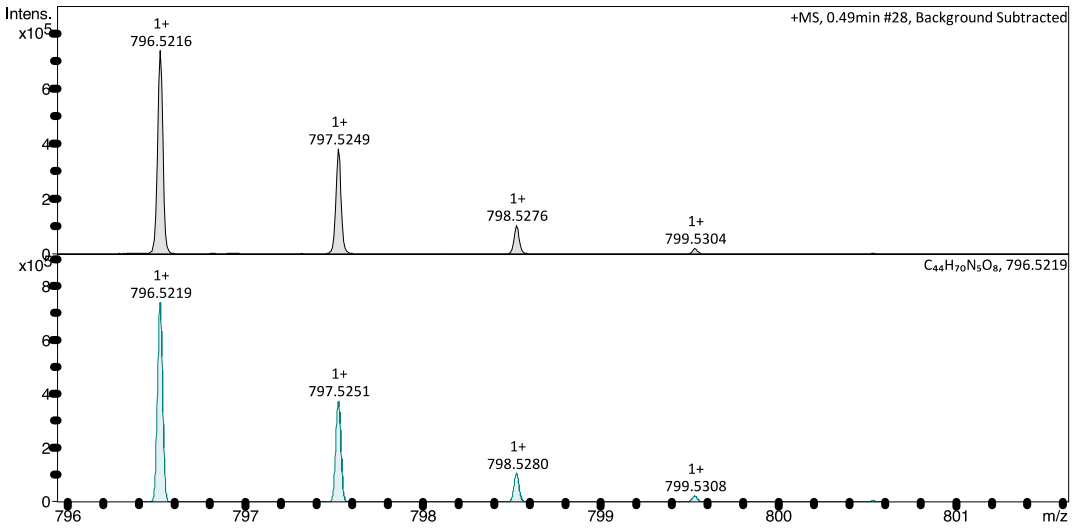
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
796.521627	1+	1	C44H70N5O8	796.521891	-0.3	4.9	12.5	even
	1+	2	C41H62N15O2	796.520543	1.4	8.0	18.5	even
	1+	3	C43H74NO12	796.520553	1.3	15.4	7.5	even
	1+	4	C56H66N3O	796.520040	2.0	54.3	25.5	even

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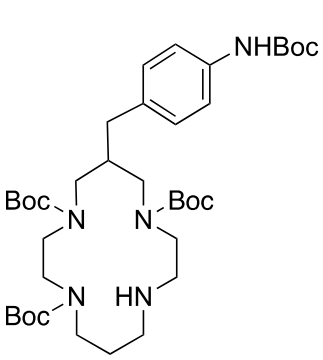
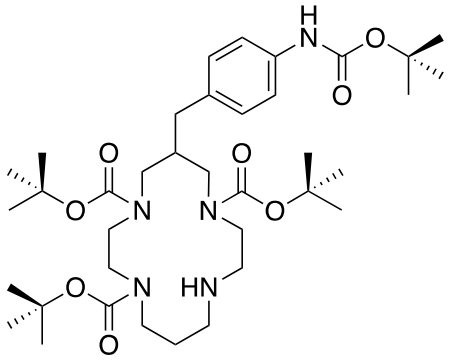
Figure S14: HRMS spectrum (ESI) of compound **5**

Compound 6





Formula: $C_{37}H_{63}N_5O_8$

Exact Mass: *705.468 g.mol^{-1}*

Molecular Weight: *705.938 g.mol^{-1}*

Description: *Yellowish oil*

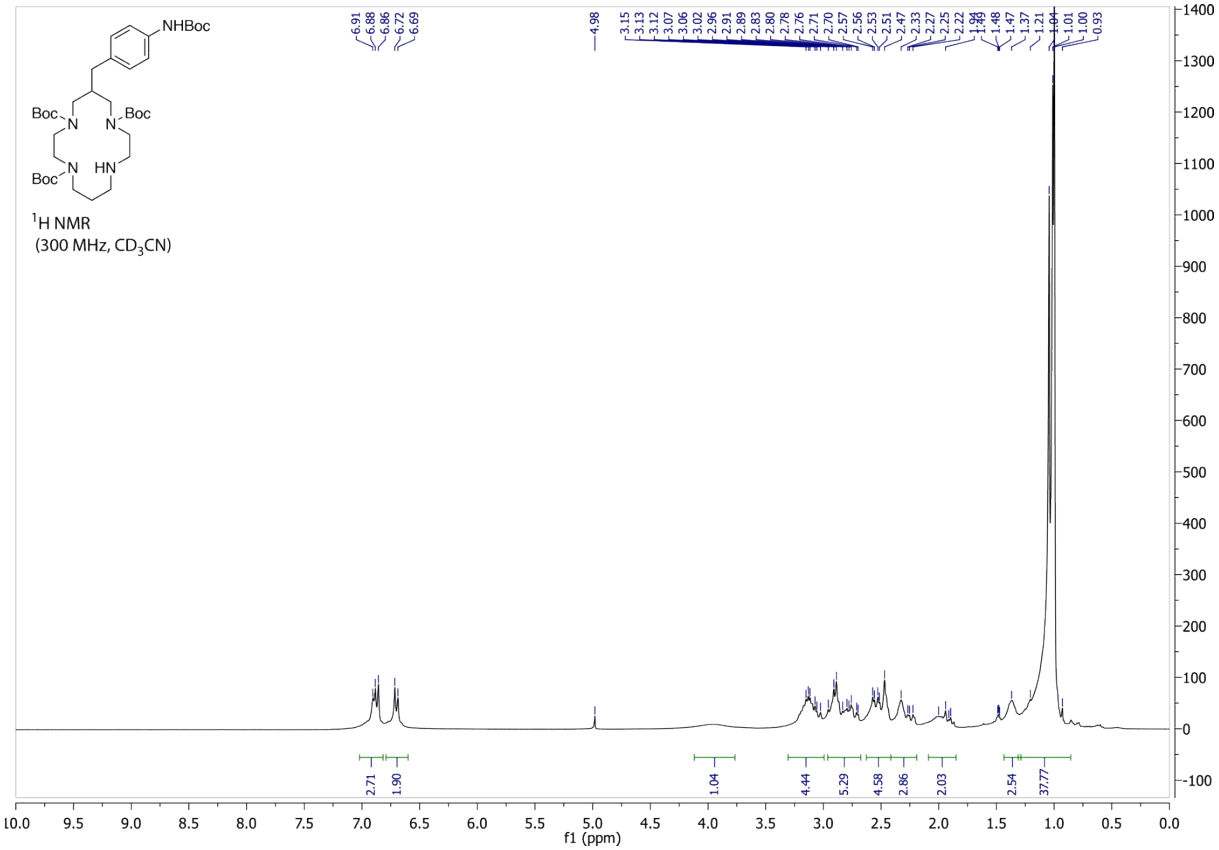


Figure S15: ^{1}H NMR spectrum (300 MHz, CD_3CN, 70°C) of compound **6**

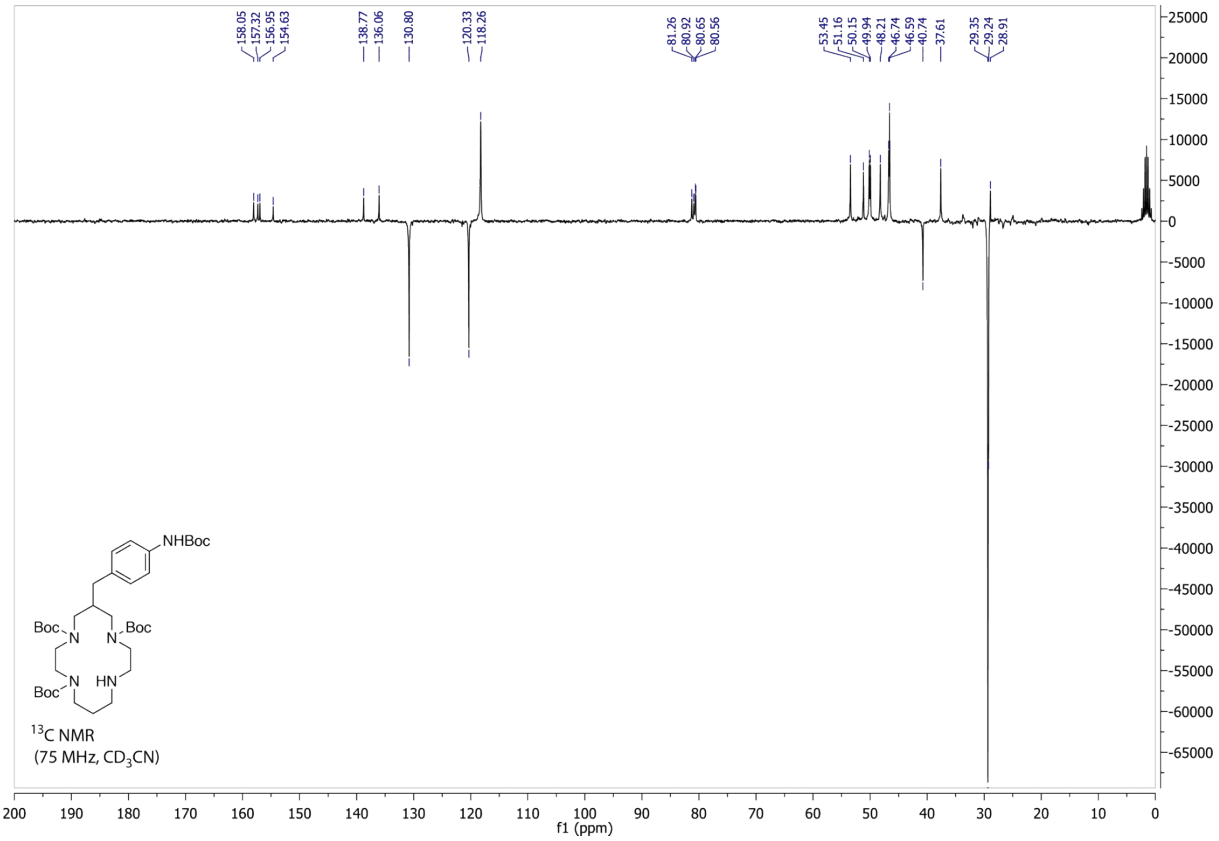


Figure S16: ^{13}C NMR spectrum (75 MHz, CD_3CN, 70°C) of compound **6**

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Plate-forme de Spectrométrie de Masse Haute Résolution

HRAM

Analysis Info

Sample Name	**triBoc-cyclam-BnNHBoc**	Acquisition Date	17/01/2018 14:17:29
Analysis Name	X039336CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
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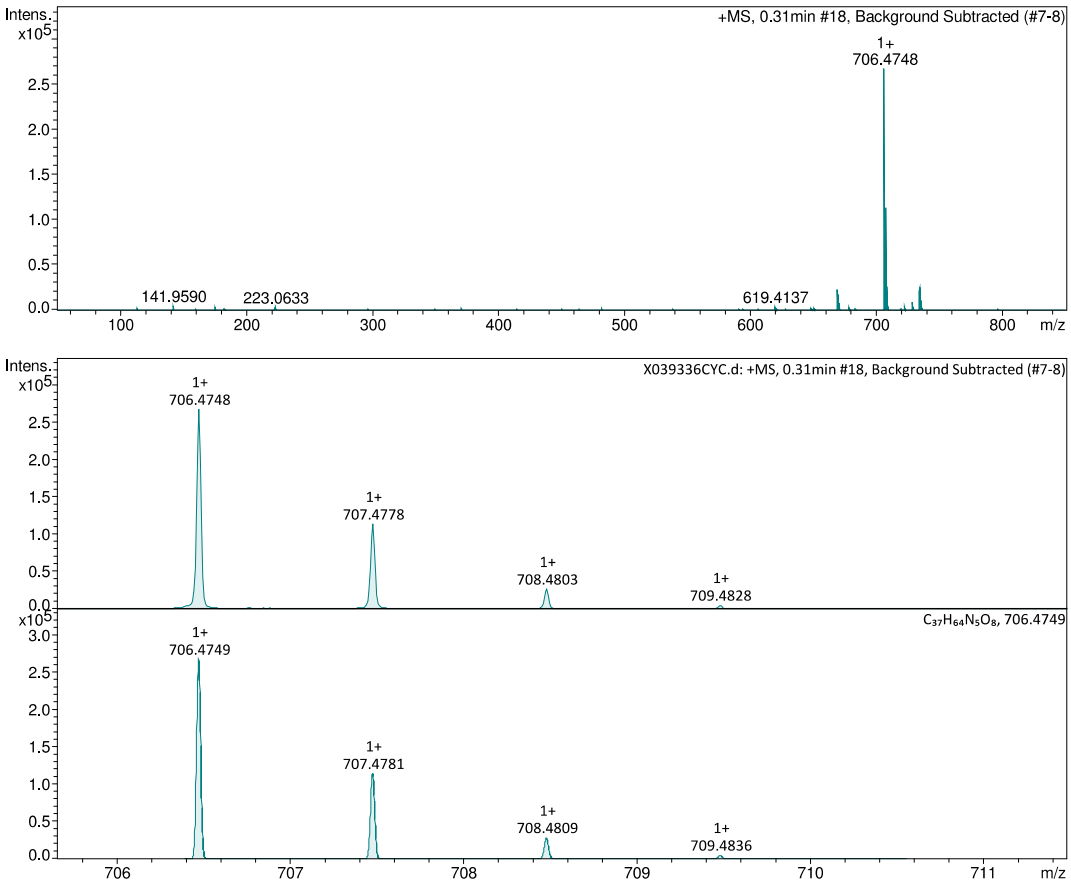
Scan End	2500 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
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	1+	2	C36H68NO12	706.473603	-1.8	10.2	4.0	even
728.456732	1+	1	C36H67NNaO12	728.455547	-1.6	11.8	4.0	even
	1+	2	C37H63N5NaO8	728.456885	0.2	19.2	9.0	even

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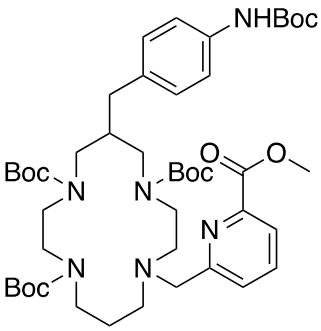
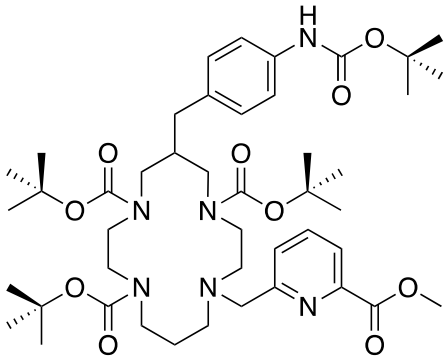
Figure S17: HMRS spectrum (ESI) of compound **6**

Compound 7





Formula: $C_{45}H_{70}N_6O_{10}$

Exact Mass: *854.515 g.mol^{-1}*

Molecular Weight: *855.087 g.mol^{-1}*

Description: *Colorless oil*

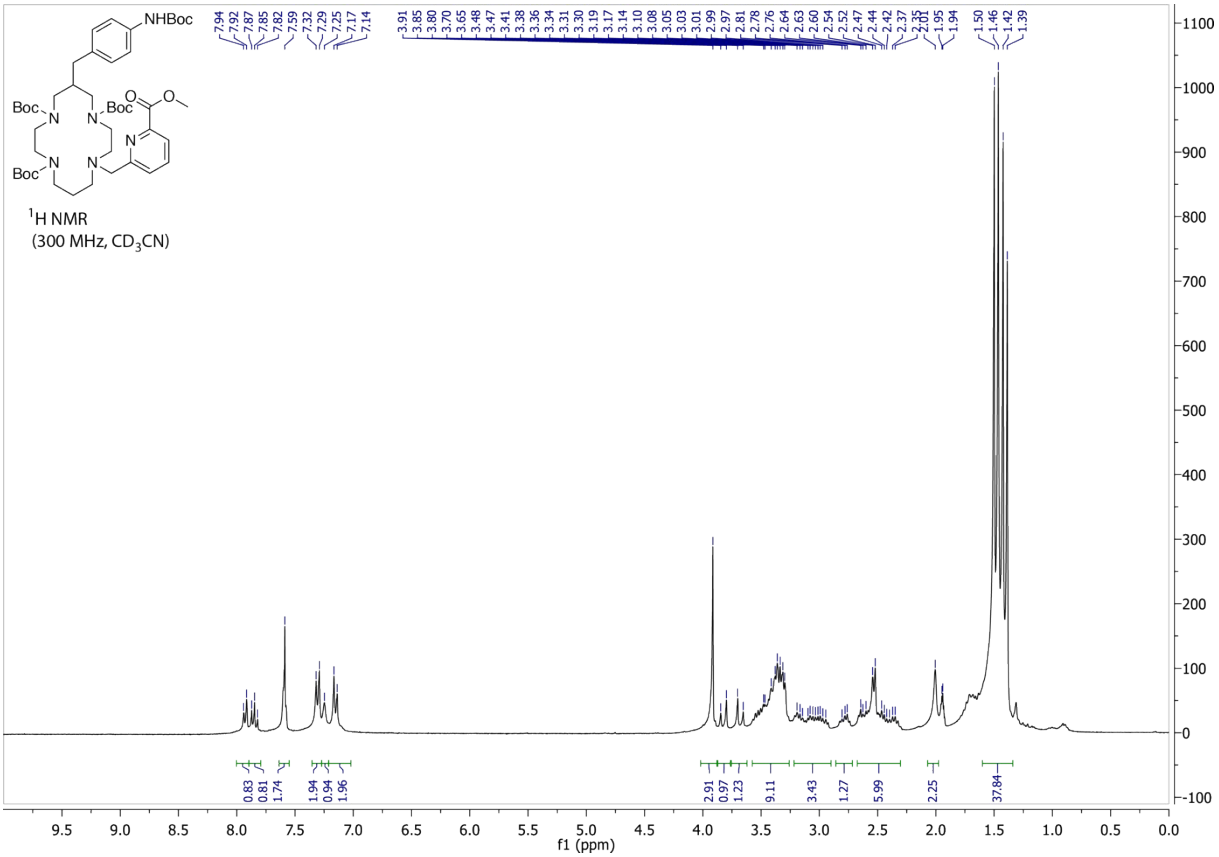


Figure S18: ^{1}H NMR spectrum (300 MHz, CD_3CN, 70°C) of compound **7**

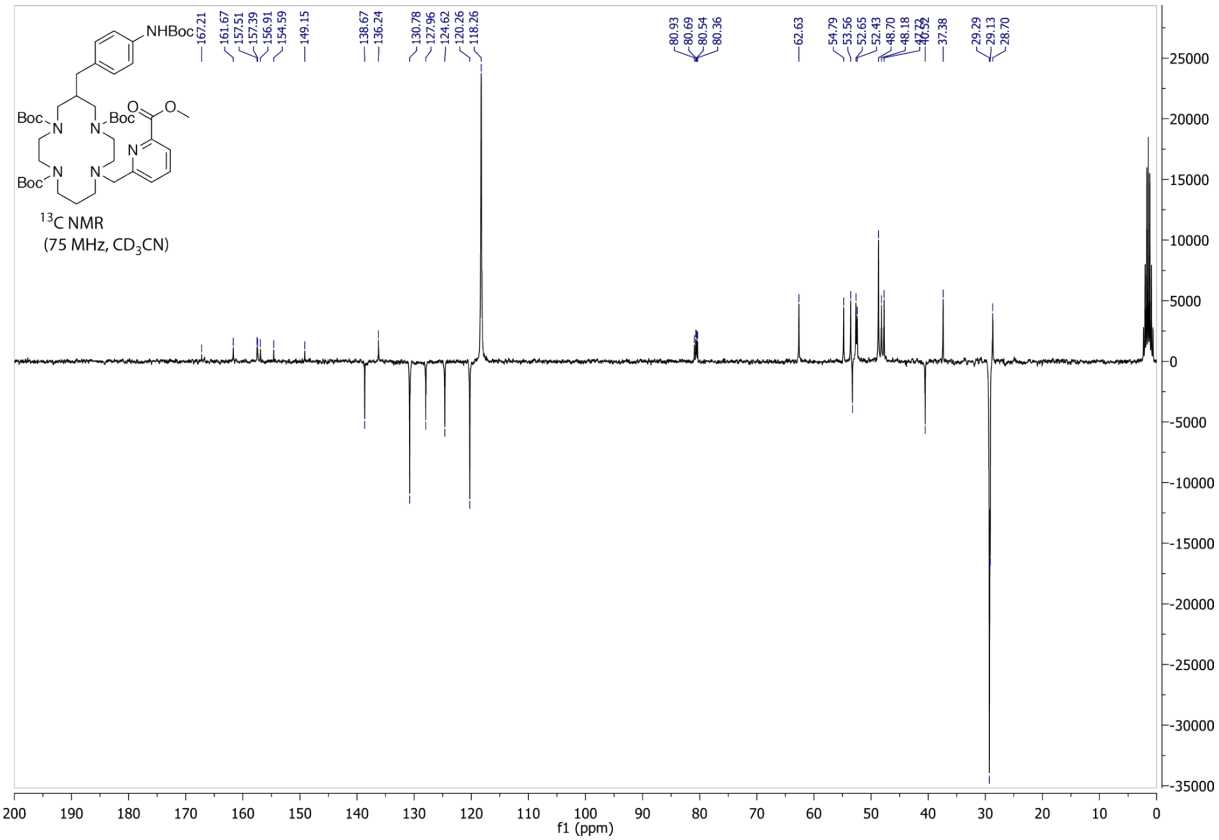


Figure S19: ^{13}C NMR spectrum (75 MHz, CD_3CN, 70°C) of compound **7**

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Plate-forme de Spectrométrie de Masse Haute Résolution

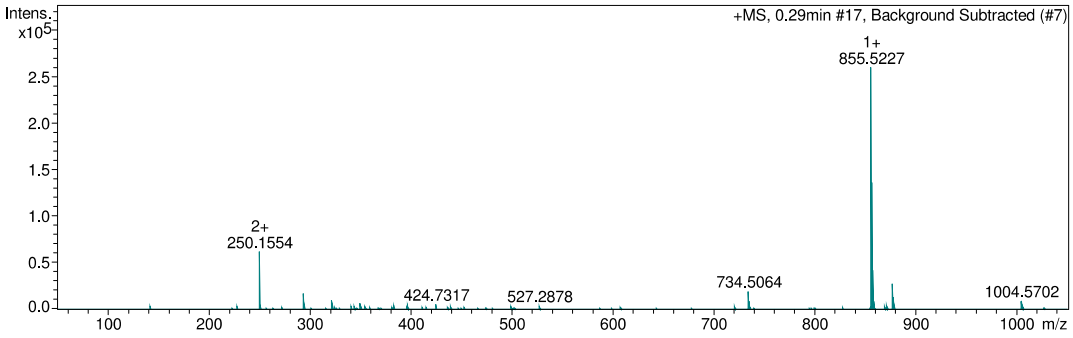
HRAM

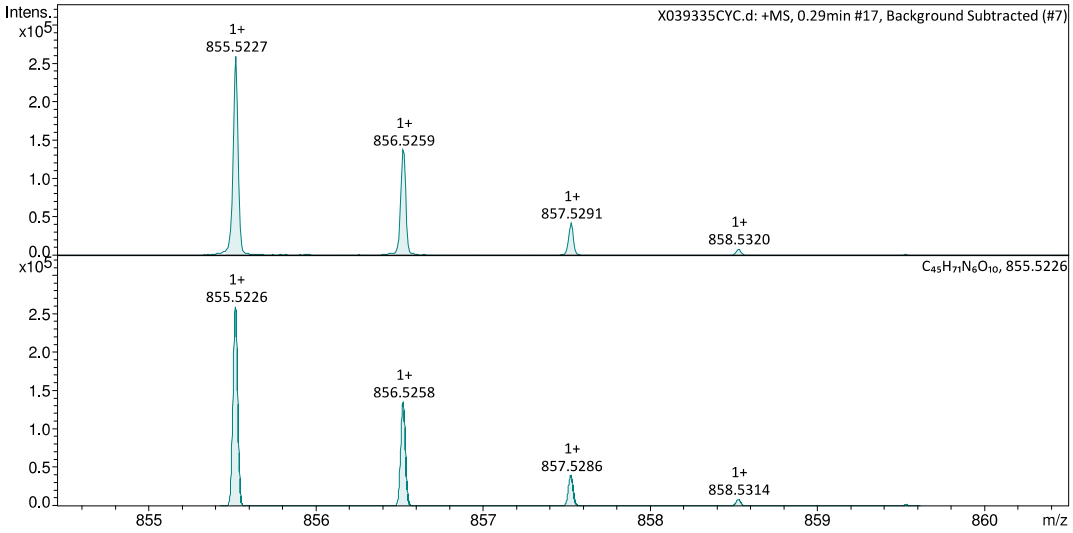
Analysis Info

Sample Name	**triBoc-cyclam-picolinate-BnNHBoc**	Acquisition Date	17/01/2018 14:16:03
Analysis Name	X039335CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan End	2500 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
250.155387	2+	1	C26H40N6O4	250.155003	-1.5	3.6	11.0	even
294.145195	2+	1	C28H40N6O8	294.144833	-1.2	8.3	13.0	even
720.490821	1+	1	C38H66N5O8	720.490591	-0.3	60.3	9.0	even
734.506405	1+	1	C39H68N5O8	734.506241	-0.2	12.0	9.0	even
	1+	2	C38H72NO12	734.504903	-2.0	14.9	4.0	even
855.522679	1+	1	C45H71N6O10	855.522619	-0.1	5.6	14.0	even
877.504944	1+	1	C45H70N6NaO10	877.504563	-0.4	20.2	14.0	even

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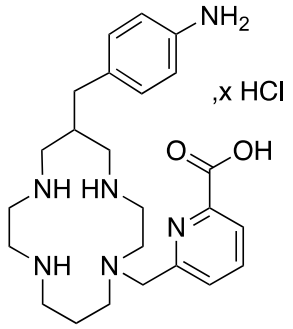
Figure S20: HRMS spectrum (ESI) of compound **7**

Compound 8 : *p*-NH_2-Bn-te1pa



Formula: $C_{24}H_{36}N_6O_2 \cdot xHCl$

Exact Mass: *440.290 g.mol^{-1}*

Molecular Weight: 440.592 *g.mol^{-1}*

Description: *Colorless oil*

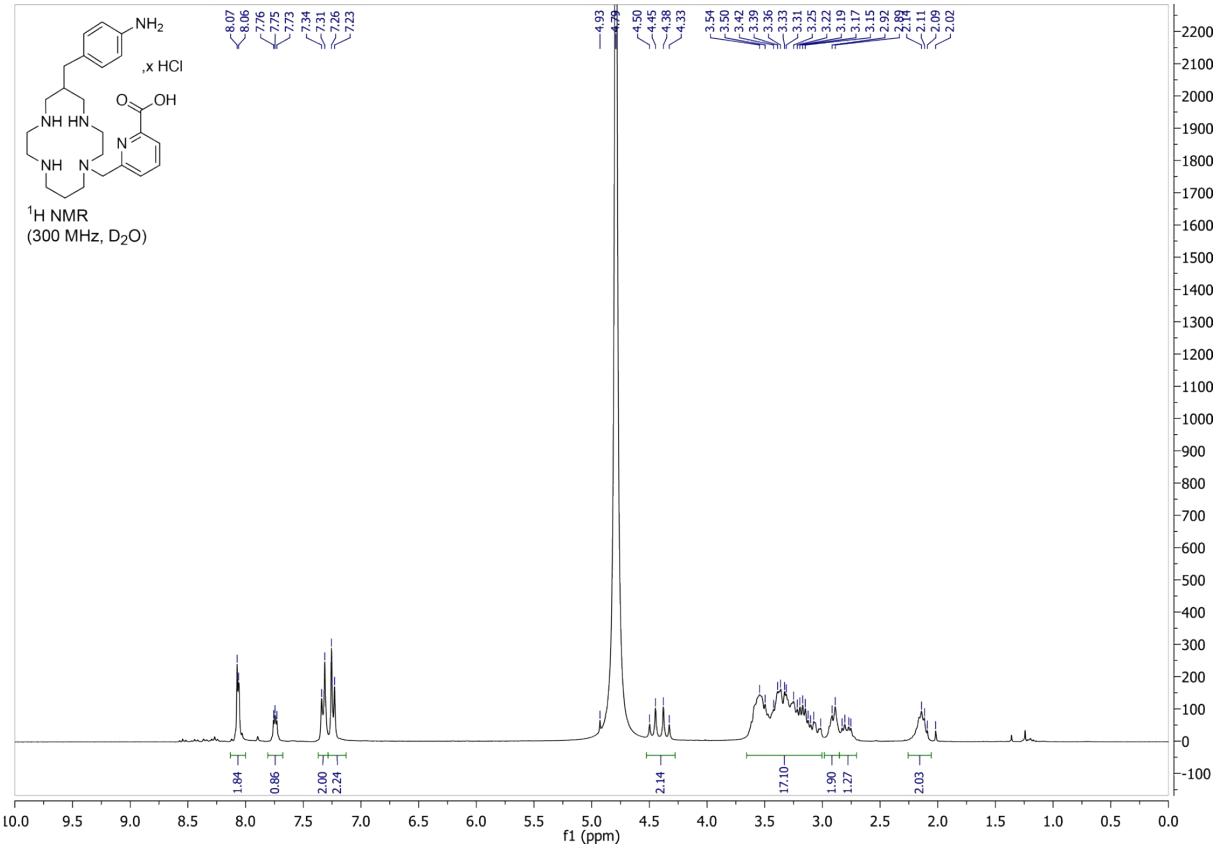


Figure S21: ^{1}H NMR spectrum (300 MHz, D_2O, 25°C) of compound **8**

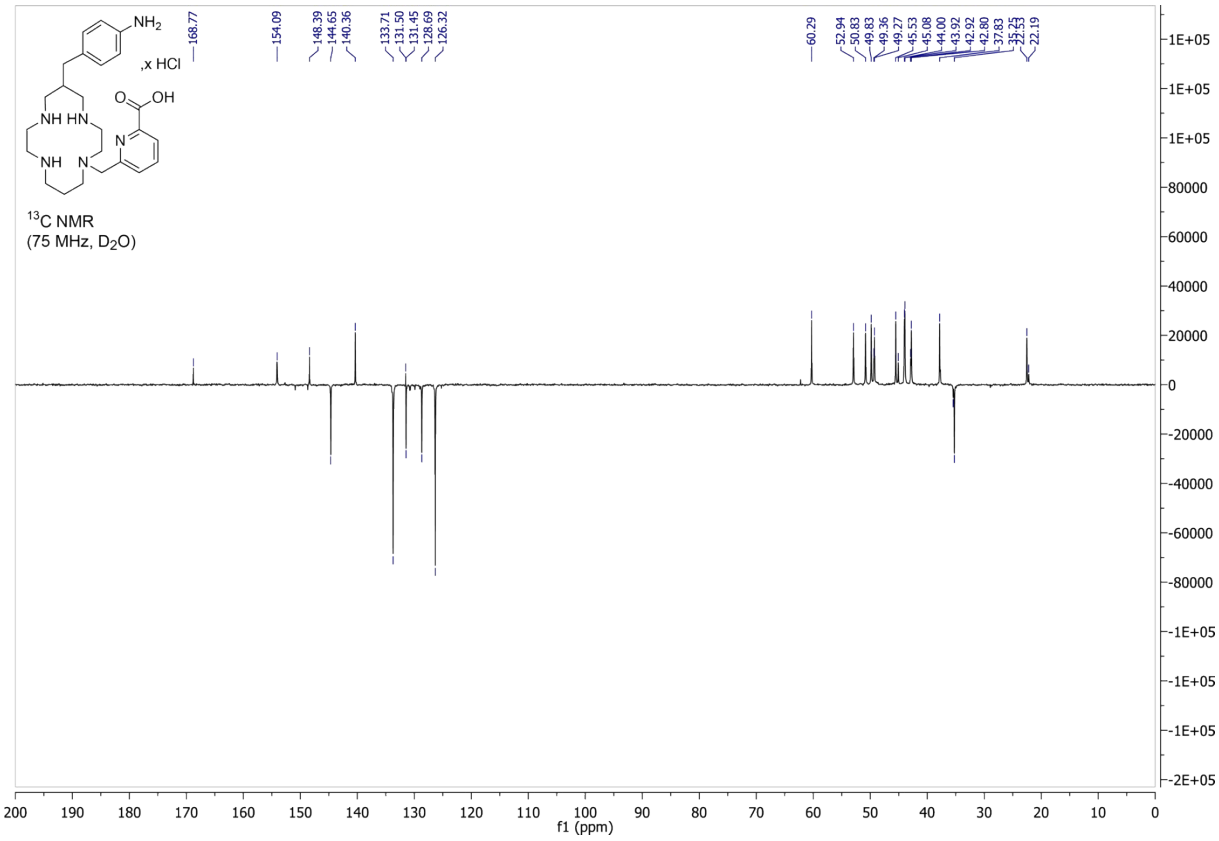


Figure S22: ^{13}C NMR spectrum (75 MHz, D_2O, 25°C) of compound **8**

HRMS *p*-SCN-Bn-TE1PA

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Plate-forme de Spectrométrie de Masse Haute Résolution

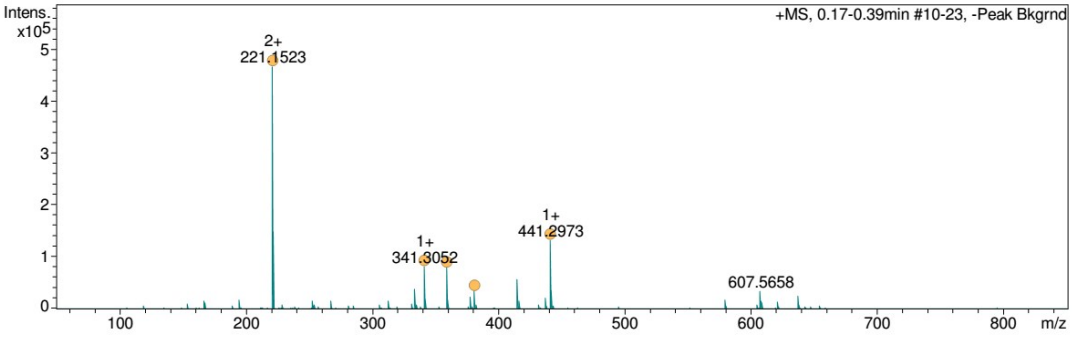
HRAM

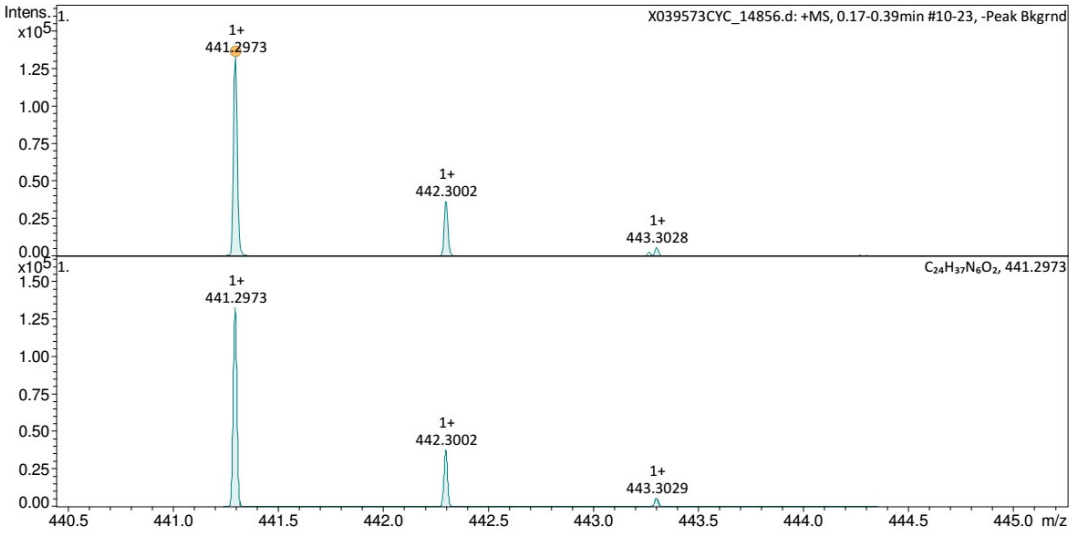
Analysis Info

Sample Name	**te1pa-BnNH2**	Acquisition Date	24/01/2018 23:15:21
Analysis Name	X039573CYC_14856.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan End	2500 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
221.152332	2+	1	C24H38N6O2	221.152264	-0.3	18.5	10.0	even
341.305177	1+	1	C21H41O3	341.305022	-0.5	6.2	2.0	even
359.315873	1+	1	C21H43O4	359.315586	-0.8	7.1	1.0	even
381.297574	1+	1	C21H42NaO4	381.297531	-0.1	1.6	1.0	even
441.297274	1+	1	C24H37N6O2	441.297251	-0.1	5.6	10.0	even

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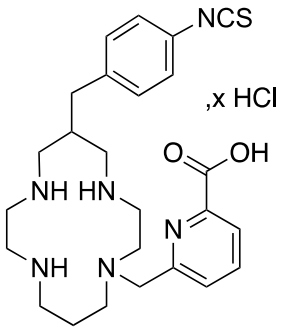
Figure S23: HRMS spectrum (ESI) of compound **9**

Compound 9 : *p*-SCN-Bn-te1pa



Formula: $C_{25}H_{34}N_6O_2S \cdot xHCl$

Exact Mass: *482.246 g.mol*$^{-1}$

Molecular Weight: 482.647 *g.mol*$^{-1}$

Description: *Colorless oil*

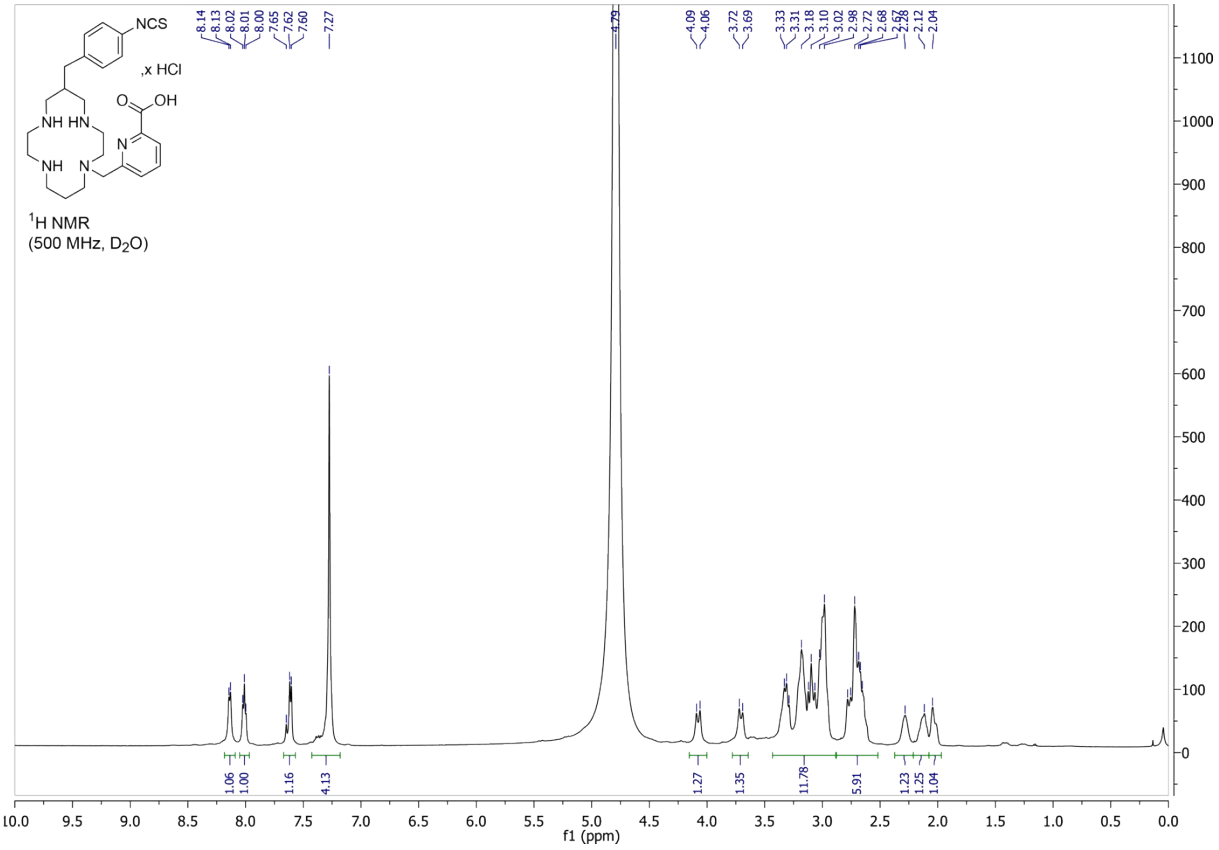


Figure S24: ^{1}H NMR spectrum (500 MHz, D_2O, 25°C) of compound **9**

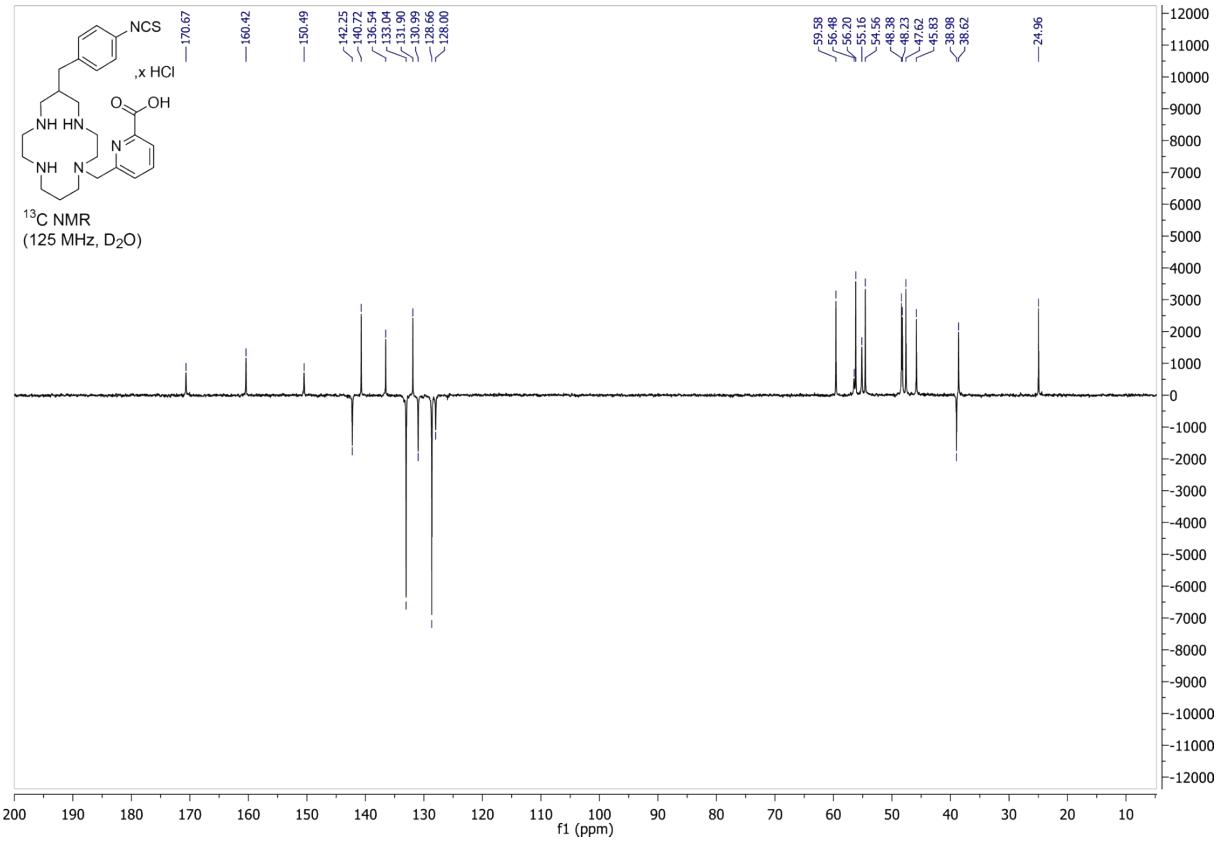


Figure S25: ^{13}C NMR spectrum (125 MHz, D_2O, 25°C) of compound **9**

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Plate-forme de Spectrométrie de Masse Haute Résolution

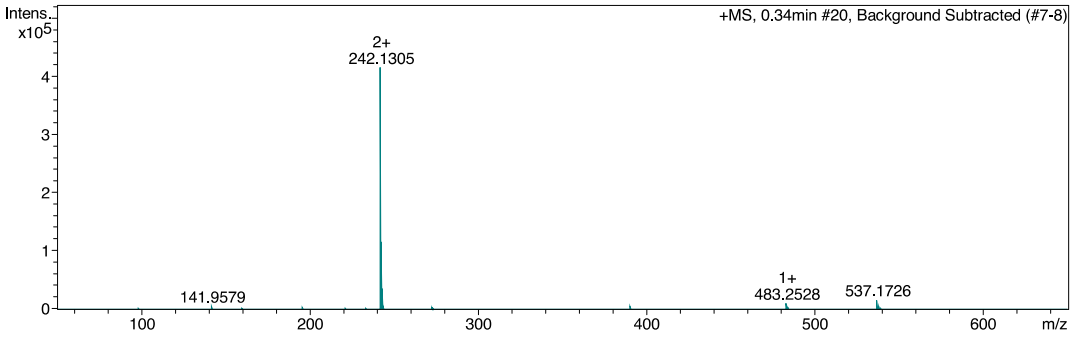
HRAM

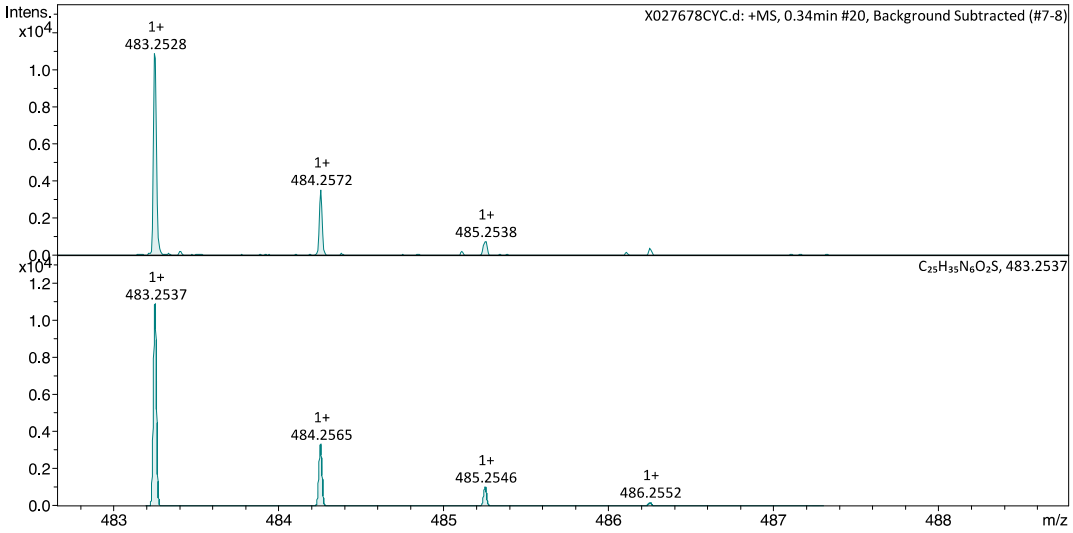
Analysis Info

Sample Name	**TLB147**	Acquisition Date	29/06/2016 14:38:51
Analysis Name	X027678CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan End	2500 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
242.130539	2+	1	C25H36N6O2S	242.130474	-0.3	14.2	12.0	even
483.252841	1+	1	C25H35N6O2S	483.253672	1.7	19.6	12.0	even
	1+	2	C24H39N2O6S	483.252334	-1.0	28.4	7.0	even
537.172577	1+	1	C25H33FeN6O2S	537.172959	0.8	13.8	13.0	even
	1+	2	C24H37FeN2O6S	537.171622	-1.7	20.3	8.0	even

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Figure S26: HRMS spectrum (ESI) of compound **9**

Table S1: Bioconjugation rates and immunoreactivity obtained with *p*-Bn-SCN-DOTA with 9E7.4

	Chelates / mAb	Immunoreactivity (%)
Exp 1	2.5	42.3 ± 3.1
Exp 2	2	39.3 ± 0.5
Exp 3	1	68.9 ± 0.3
Exp 4	1	69.9 ± 0.3
Exp 5	2	53.2 ± 0.7

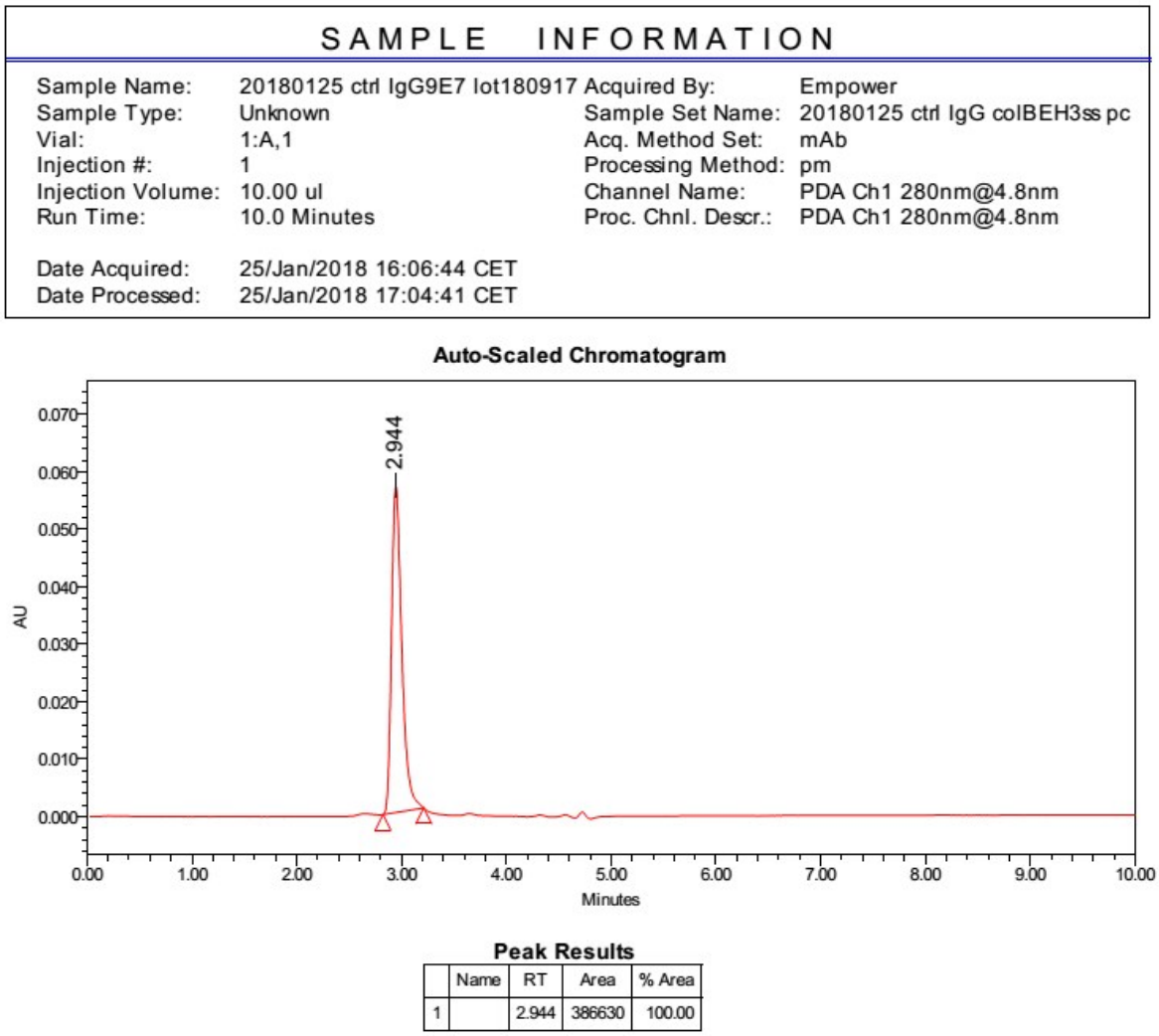
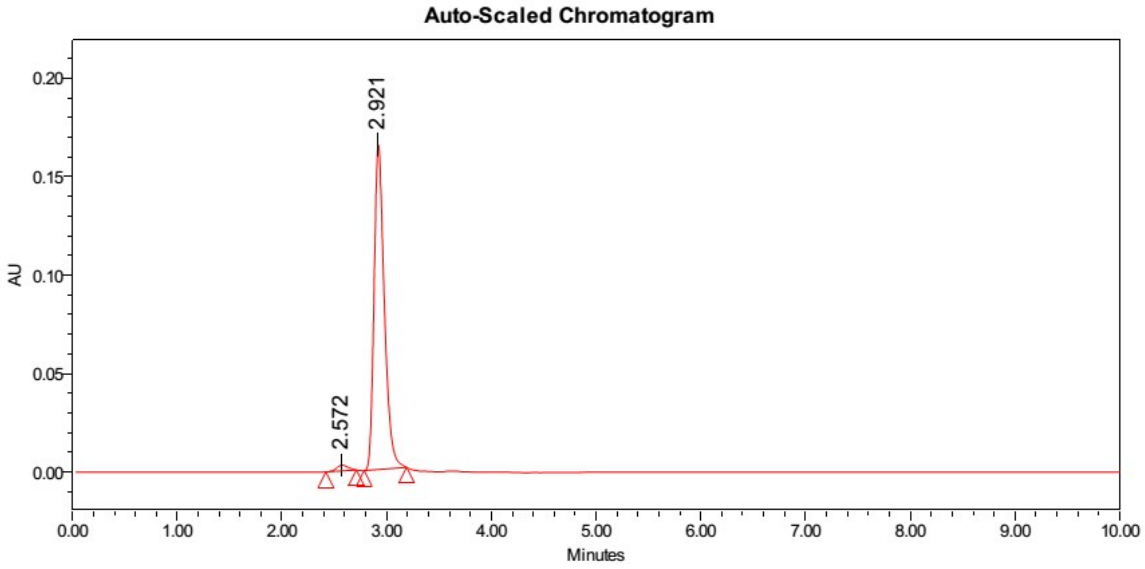


Figure S27: UPLC profile of unmodified mAb 9E7.4

SAMPLE INFORMATION

Sample Name:	20180125 ctrl IgG9E7NOTA	Acquired By:	Empower
Sample Type:	Unknown	Sample Set Name:	20180125 ctrl colBEH3 ss preco
Vial:	1:A,1	Acq. Method Set:	mAb
Injection #:	1	Processing Method:	pm
Injection Volume:	5.00 ul	Channel Name:	PDA Ch1 280nm@4.8nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 280nm@4.8nm
Date Acquired:	25/Jan/2018 15:20:12 CET		
Date Processed:	25/Jan/2018 16:08:52 CET		



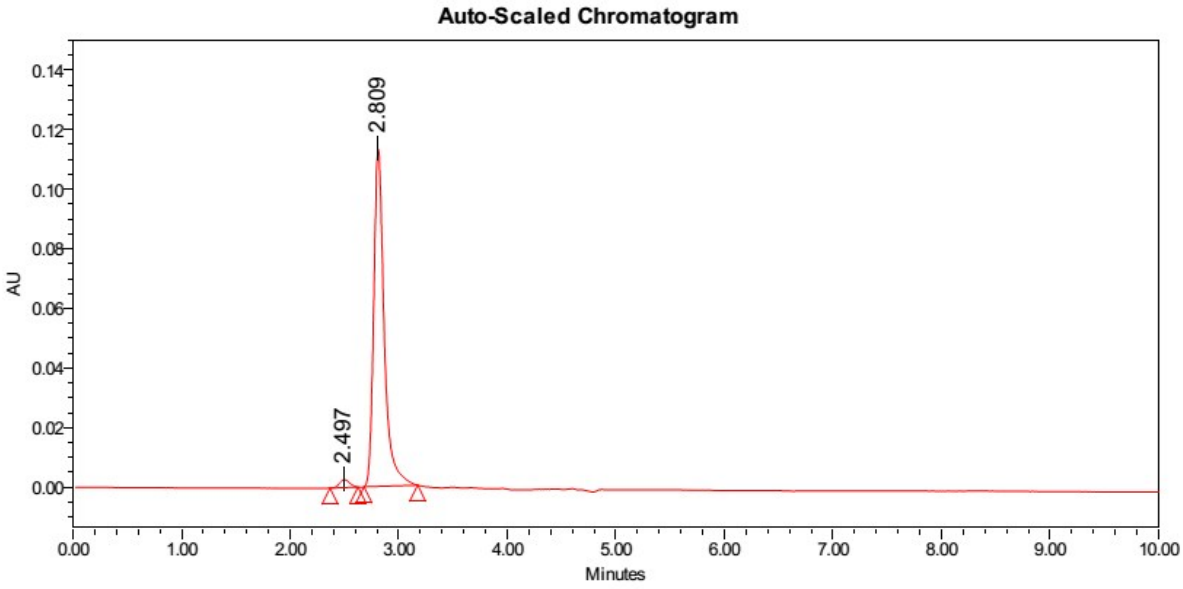
Peak Results

	Name	RT	Area	% Area
1		2.572	22014	1.92
2		2.921	1124214	98.08

Figure S28: UPLC profile of 9E7.4-*p*-SCN-Bn-NOTA

SAMPLE INFORMATION

Sample Name:	20170217 ctrl IgG9E7-DOTA	Acquired By:	Empower
Sample Type:	Unknown	Sample Set Name:	20170217 ctrl mAb couple ASN
Vial:	1:A,1	Acq. Method Set:	mAb
Injection #:	1	Processing Method:	pm
Injection Volume:	5.00 ul	Channel Name:	PDA Ch1 280nm@4.8nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 280nm@4.8nm
Date Acquired:	17/Feb/2017 15:38:35 CET		
Date Processed:	20/Jun/2017 17:38:16 CEST		



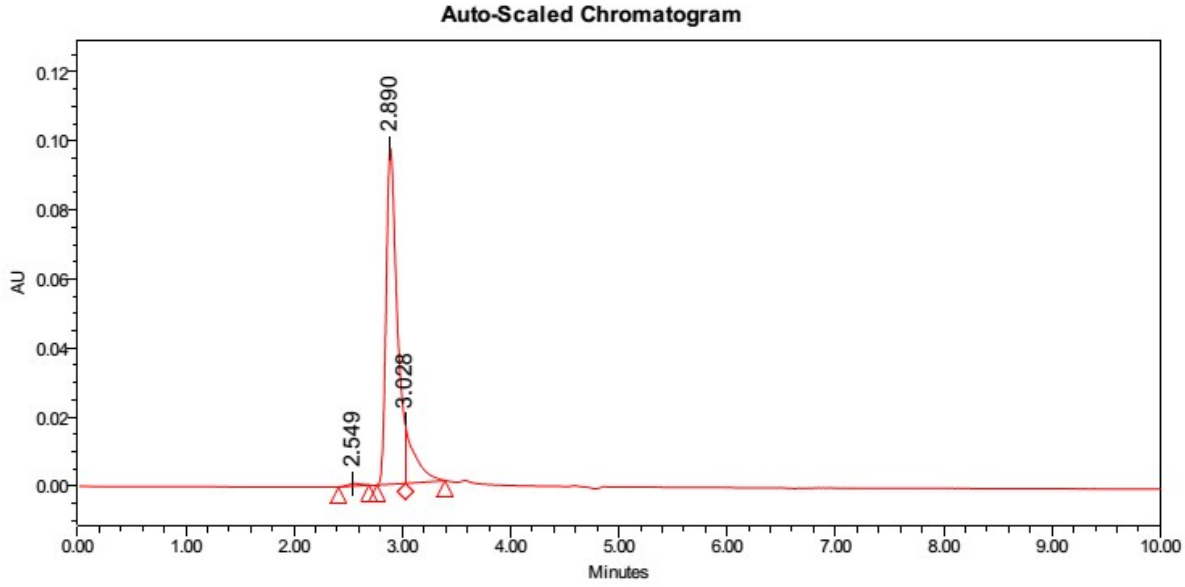
Peak Results

	Name	RT	Area	% Area
1		2.497	17436	2.24
2		2.809	760578	97.76

Figure S29: UPLC profile of 9E7.4-*p*-SCN-Bn-DOTA

SAMPLE INFORMATION

Sample Name:	20170217 ctrl IgG9E7-HTE1PA	Acquired By:	Empower
Sample Type:	Unknown	Sample Set Name:	20170217 ctrl mAb couple ASN
Vial:	1:A,2	Acq. Method Set:	mAb
Injection #:	1	Processing Method:	pm
Injection Volume:	5.00 ul	Channel Name:	PDA Ch1 280nm@4.8nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 280nm@4.8nm
Date Acquired:	17/Feb/2017 15:49:16 CET		
Date Processed:	20/Jun/2017 17:38:44 CEST		



Peak Results

	Name	RT	Area	% Area
1		2.549	5868	0.74
2		2.890	676559	85.87
3		3.028	105421	13.38

Figure S30: UPLC profile of 9E7.4-*p*-SCN-Bn-TE1PA