Supporting Information

Mechanostereoselective one-pot synthesis of functionalized head-to-head cyclodextrin [3]rotaxanes and their application as Magnetic Resonance Imaging contrast agents

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General procedures

General Analytical and Synthetic Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 or 600 MHz on BrukerAvance III spectrometers. Chemical shifts (δ) are reported in ppm from tetramethylsilane using residual solvent peaks for calibration. Mass spectrometry was performed by ESI on a BrukermicroTOF spectrometer.All chemicals were purchased from commercial suppliers and used without further purification. Flash column chromatography was performed using silica gel from Merck (40-63 µm) or GraceResolv High Resolution Flash Cartridges (particle size 40 µm). Thin layer chromatography was performed using aluminium plates pre-coated with silica gel 60 F254 0.20 mm layer thickness purchased from VWR. Absorption spectra were recorded on a JASCO V-670 spectrophotometer.

Relaxivity profiles. Proton NMRD (nuclear magnetic relaxation dispersion) profiles were recorded on a StelarSMARtracer Fast Field Cycling NMR relaxometer (0.01-10 MHz) and a Bruker WP80 NMR electromagnet adapted to variable field measurements and controlled by a SMARtracer PC-NMR console. The temperature was monitored by a VTC91 temperature control unit and maintained by a gas flow. The temperature was determined by previous calibration with a Pt resistance temperature probe. The longitudinal relaxation rates ($1/T_1$) were determined in water. The least-square fit of the ^{1}H NMRD data was performed by using MicroMath Scientist version 2.0 (Salt Lake City, UT, USA).

ICP-OES Measurements. The exact contents in Gd^{3+} ion of the solutions used for the relaxivity profiles were determined by Induced Coupled Plasma Optical Emission Spectrometer (ICP-OES) measurements. These measurements were performed on a JobinYvon ULTIMA2 Spectrometer. Both standard solutions of Gd^{3+} and the samples were prepared in 5% HNO_3 and the 342.246 nm band for Gd^{3+} (the most accurate of the three Gd^{3+} band) was used. All the measurements were repeated at least three times.

Biodistribution. Biodistribution studies were conducted *in vivo* by MRI to assess the uptake and clearance from kidney, muscle, spleen, liver more easily observable by MRI.

All animal work was performed in accordance with the institutional animal protocol guidelines in place at the University Paris Descartes, saisine CEEA34.JS.142.1 and approved by the Institute's Animal Research Committee.

Wild-type female 8 weeks BALB/c mice were anaesthetized by Isoflurane (1,5% air/O_2 0,5/0,25 $L.min^{-1}$) inhalation and placed in a dedicated contention cradle. 100 µL of Gadolinium rotaxanes in saline 0,9% with an optimized concentration of 10 mM of Gd were intravenously injected via the tail vein while the mouse was in the scanner. For reproductibility studies, 6 mice were injected per contrast agents (n=6, 3 contrast agents, 18 mice).

Images were acquired at 7 Tesla (300 MHz) micro imaging spectrometer, equipped with a ^{1}H radiofrequency linear coil of 4 cm inner diameter (Bruker, Karlsruhe Germany)

The scanning protocol was developed using Paravision 5.1 software.

DCE Dynamic contrast Enhanced sequence was recorded using Intragate Flash multislices for motion free artifacts T1 weighted sequence. The final images have a spatial resolution of 117 µm x 117 µm in plane. The total scan time was in the order of 3 min 14 s per images.

The dynamic follow-up is measured during in a scan time of 40 min.

To study the biodistribution of the rotaxanes, several regions of interest (ROI) were monitored, the liver and the kidney. The corresponding MRI intensity related to the amount of the rotaxane contrast agent was plotted against time to visualize the uptake and clearance of scaffolds in the organs.The intensitylevel of the tissue wasalsocheckedthanks to a phantom tube filledwith saline as reference. The percentage of enhancement of the kidney or liver tissue canthereforebecompared to the pre-contrast image.Comparison with commercial DOTA-Gd (Guebert, France) as a reference was also performed at the corresponding concentration of Gd (10mM).

Syntheses

Functionalized cyclodextrins**7a-b** and **8a-b** were synthesized according to literature[1]

1,12-diazidododecane (3)[2]

Under argon, a solution of 1,12-dibromododecane (0.5 g, 1.5 mmol) and sodium azide (0.4 g, 6.2 mmol) in dry dimethylformamide (5 ml) was stirred at 80 °C for 24 h. Then, the solvent was removed under reduced pressure and water (50 ml) was added. The product was extracted with dichloromethane (150 ml). The organic layer was dried over $MgSO_4$ and filtered. Evaporation of the filtrate yielded a brown oil (380 mg, 99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.26 (t, *J* = 7.0 Hz, 4H), 1.65 – 1.55 (q, *J* = 7.1 Hz, 4H), 1.42 – 1.25 (m, 16H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 51.65, 29.63, 29.60, 29.29, 28.99, 26.87.

Dimethyl 5-(propargyloxy)isophthalate (4)[3]

In a round bottom flask was added dimethyl-5-hydroxyisophthalate (300 mg, 1.43 mmol), 3 mL of anhydrous DMF and K_2CO_3 (395 mg, 2.86 mmol). Propargylbromide (80% in toluene) (293 µL, 2.14 mmol) was added drop-wise. The reaction was stirred at room temperature for 15 hours under nitrogen. It was poured in 10 mL of water. A white precipitate was formed. It was filteredand washed with water to obtain the product as a white solid (290 mg, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (t, *J* = 1.4 Hz, 1H), 7.83 (d, *J* = 1.4 Hz, 2H), 4.78 (d, *J* = 2.4 Hz, 2H), 3.94 (s, 6H), 2.55 (t, *J* = 2.4 Hz, 1H)

5-(propargyloxy)isophthalic acid (5)[3]

In a round bottom flask was added dimethyl 5-(propargyloxy)isophthalate **4**(290 mg, 1.16 mmol), 2 mL of THF, 2 mL of water, 2 mL of MeOH and NaOH (285 mg, 5.82 mmol). The reaction was stirred

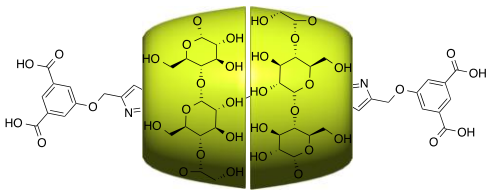
at 70°C for 3 hours. THF and MeOH were evaporated from the mixture. 10 mL of HCl 1M was added. The white precipitate was filteredand washed with water to obtain the product (240 mg, 94%).

^{1}H NMR (400 MHz, DMSO) δ 13.33 (s, 2H), 8.11 (t, *J* = 1.5 Hz, 1H), 7.72 (d, *J* = 1.5 Hz, 2H), 4.95 (d, *J* = 2.4 Hz, 2H), 3.63 (t, *J* = 2.3 Hz, 1H)

^{13}C NMR (101 MHz, DMSO) δ 166.29, 157.32, 132.64, 122.91, 119.62, 78.94, 78.67, 55.98

Synthesis of [3]rotaxanes

Compound CD [3]rotaxane (6)



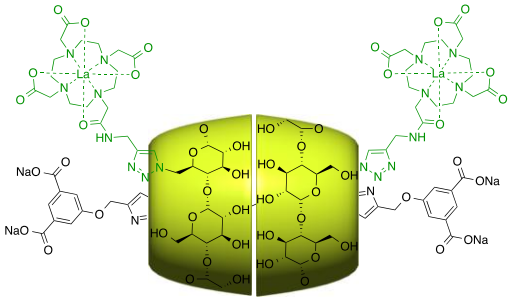
A mixture of 1,12-diazidododecane **3**(30 mg, 0.12 mmol) andα-CD (231 mg, 0.24 mmol) in distilled water (2.5 mL) was stirred at room temperature for 15 min under inert atmosphere. Then, K_2CO_3 (98 mg, 0.71 mmol), 5-(propargyloxy)isophthalate**5** (54 mg, 0.26 mmol), $CuSO_4{\cdot}5H_2O$ (18 mg, 0.07 mmol), PMDETA (25 µL, 0.12 mmol) and sodium ascorbate (28 mg, 0.14 mmol) were added. The reaction mixture was stirred for 15 hours at room temperature under inert atmosphere. HCl 1M was poured in the reaction. The precipitate was filteredand washed with water and acetone. MeOH was added to dissolve the solid and then water was added. The precipitate was filteredagain and washed with water and acetone. The last procedure was repeated 2 times to yield a white powder (47 mg, 30%).

^{1}H NMR (600 MHz, DMSO) δ 13.33 (s, 4H), 8.11 (s, 2H), 8.09 (s, 2H), 7.72 (s, 4H), 5.78 (s, 12H), 5.55 (s, 12H), 5.18 (d, *J* = 4.6 Hz, 4H), 4.77 (s, 12H), 4.45 (s, 12H), 4.25 (s, 2H), 4.09 (s, 2H), 3.76 (m, 12H), 3.62 (t, *J* = 9.5 Hz, 24H), 3.49 (d, *J* = 10.8 Hz, 12H), 3.43 (m, 12H), 3.24 (t, *J* = 7.7 Hz, 12H), 3.17 (s, 6H), 1.85 (d, *J* = 10.2 Hz, 4H), 1.49 – 1.32 (m, 16H).

^{13}C NMR (151 MHz, DMSO) δ 206.49, 166.36, 158.34, 141.72, 132.64, 123.70, 122.58, 119.07, 102.15, 81.60, 73.38, 72.20, 71.99, 61.79, 59.39, 49.90, 48.60, 31.17, 30.70, 30.67, 30.33, 27.44

HRMS (ESI):*m/z* calc $[M-2H]^{2-}$ ($C_{106}H_{158}N_6O_{70}$) 1317.4500 ; found 1317.4431.

Compound RotLa$_2$ (1a)



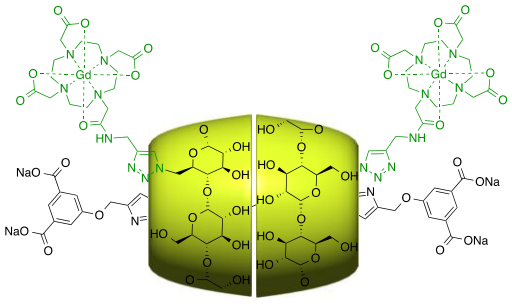
A solution of diazidododecane **3**(10 mg, 0.04 mmol) and CD-La**7a**(125 mg, 0.08 mmol) in distilled water (1.5 ml) was stirred under argon until the solution became cloudy (15 minutes). Then, 5-(ethynyloxy)isophthalic acid **5** (18 mg, 0.09 mmol), sodium bicarbonate (13 mg, 0.16 mmol),copper(II) sulfate pentahydrate (6 mg, 0.024 mmol), N,N,N′,N″,N″-Pentamethyldiethylenetriamine (7 mg, 8 µl, 0.04 mmol) and sodium ascorbate (10 mg, 0.05 mmol) was added. The solution was stirred at room temperature under argon for 24 hours. Water was removed. The solid was purified by size-exclusion chromatography in water to yield the rotaxane (61 mg, 39%).

^{1}H NMR (400 MHz, D_2O) δ 8.26 (s, 2H, triaz-cap/axis), 7.95 (s, 2H, H_{para}arom. cap), 7.88 (s, 2H, triaz-CD), 7.63 (s, 4H, H_{ortho} arom. cap), 5.39 (s, 4H, CH_2-cap), 5.19 (s, 2H), 5.10 (d, J = 2.68 Hz, 2H anomeric), 5.09 – 5.00 (m, 10H, CD anomeric), 4.60 – 4.29 (m, 10H), 4.01 – 3.46 (m, 72H), 3.33 – 2.20 (m, 48H), 2.10 – 1.80 (s broad, 4H, axis), 1.65 – 1.30 (s broad, 16H, -CH_2- axis).

^{13}C NMR (151 MHz, D_2O) δ 175.27, 143.57, 143.08, 126.16, 124.78, 102.63, 102.53, 102.48, 102.44, 102.40, 102.19, 80.94, 80.89, 80.72, 73.92, 73.40, 72.69, 72.26, 72.21, 71.94, 71.90, 71.83, 71.64, 60.95, 59.86, 59.75, 59.66, 59.57, 58.92, 50.76, 48.86, 42.92, 34.93, 31.51, 31.31, 27.95.

HRMS (ESI):*m/z* calc $[M-3H]^{3-}$ ($C_{144}H_{211}La_2N_{22}O_{82}$) 1279.3720 ; found 1279.3663.

Compound RotGd$_2$ (1b)

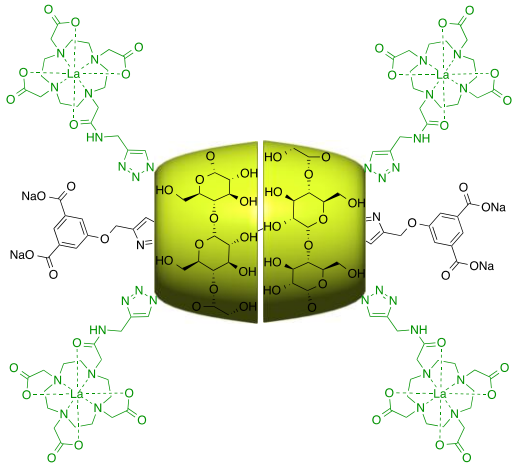


A solution of diazidododecane **3**(10 mg, 0.04 mmol) and CD-Gd**7b**(126 mg, 0.08 mmol) in distilled water (1.5 ml) was stirred under argon until the solution became cloudy (15 minutes). Then, 5-(ethynyloxy)isophthalic acid **5** (18 mg, 0.09 mmol), sodium bicarbonate (13 mg, 0.16 mmol), copper(II) sulfate pentahydrate (6 mg, 0.02 mmol), N,N,N′,N″,N″-Pentamethyldiethylenetriamine (7 mg, 8 µl, 0.04 mmol) and sodium ascorbate (10 mg, 0.05 mmol) was added. The solution was stirred

at room temperature under argon for 24 hours. Water was removed. The solid was purified by size-exclusion chromatography in water to yield the rotaxane (55 mg, 36%).

HRMS (ESI):*m/z* calc $[M-3H]^{3-}$ ($C_{144}H_{211}Gd_2N_{22}O_{82}$) 1292.0521 ; found 1292.0485.

Compound RotLa$_4$ (2a)



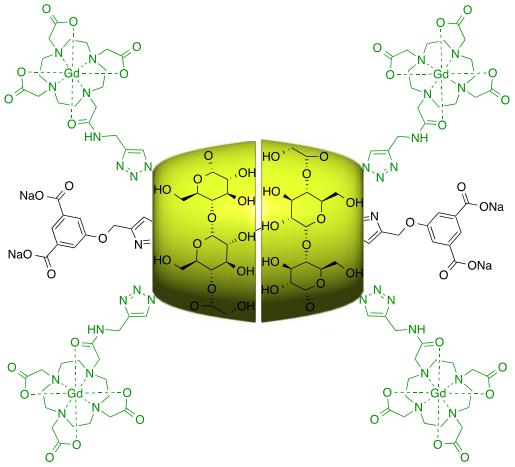
A solution of diazidododecane **3**(5.8 mg, 0.02 mmol) and CD-La$_2$**8a**(100 mg, 0.05 mmol) in distilled water (1.0 ml) was stirred under argon until the solution became cloudy (15 minutes). Then, 5-(ethynyloxy)isophthalic acid **5** (10.4 mg, 0.05 mmol), sodium bicarbonate (7.7 mg, 0.09 mmol), copper(II) sulfate pentahydrate (3.4 mg, 0.01 mmol), PMDETA (4 mg, 5 µl, 0.02 mmol) and sodium ascorbate (5.5 mg, 0.028 mmol) was added. The solution was stirred at room temperature under argon for 24 hours. Water was removed. The solid was purified by size-exclusion chromatography to yield the rotaxane (43 mg, 37%).

^{1}H NMR (400 MHz, D_2O) δ 8.29 (s, 2H, triaz-cap/axis), 7.92 (s, 6H, 4H triaz-CD, 2H$_{para}$arom. cap), 7.56 (s, 4H, H$_{ortho}$ arom. cap), 5.38 (m, 4H, CH_2-cap), 5.19 (s, 4H), 5.08 (s, 10H), 4.70 – 4.33 (m, 16H), 4.07 – 3.44 (m, 88H), 3.40 - 2.26 (m, 92H), 2.08 – 1.92 (s broad, 4H), 1.48 (s, 16H, -CH_2- axis).

^{13}C NMR (151 MHz, D_2O) δ 179.52, 179.17, 175.35, 173.38, 157.46, 143.28, 138.35, 126.19, 122.64, 117.60, 102.67, 102.36, 80.94, 80.75, 73.88, 73.36, 72.63, 72.04, 71.86, 71.63, 61.05, 59.80, 59.22, 50.86, 34.97, 31.44, 31.33, 31.22, 30.24, 28.09.

HRMS (ESI):*m/z* calc $[M-3H]^{3-}$ ($C_{182}H_{265}La_4N_{38}O_{94}$) 1680.7798 ; found 1680.7815.

Compound RotGd$_4$ (2b)



A solution of diazidododecane**3**(5.8 mg, 0.02 mmol) and CD-Gd$_2$**8b**(101 mg, 0.05 mmol) in distilled water (1.0 ml) was stirred under argon until the solution became cloudy (15 minutes). Then,5-(ethynyloxy)isophthalic acid **5** (10.0 mg, 0.049 mmol), sodium bicarbonate (7.8 mg, 0.09 mmol), copper(II) sulfate pentahydrate (3.4 mg, 0.01 mmol), PMDETA (4 mg, 5 µl, 0.02 mmol) and sodium ascorbate (5.6 mg, 0.03 mmol) was added. The solution was stirred at room temperature under argon for 24 hours. Water was removed. The solid was purified by size-exclusion chromatography (61 mg, 52%).

HRMS (ESI):*m/z* calc $[M-3H]^{3-}$ ($C_{182}H_{265}Gd_4N_{38}O_{94}$) 1706.1392 ; found 1706.1448.

DOSY spectra

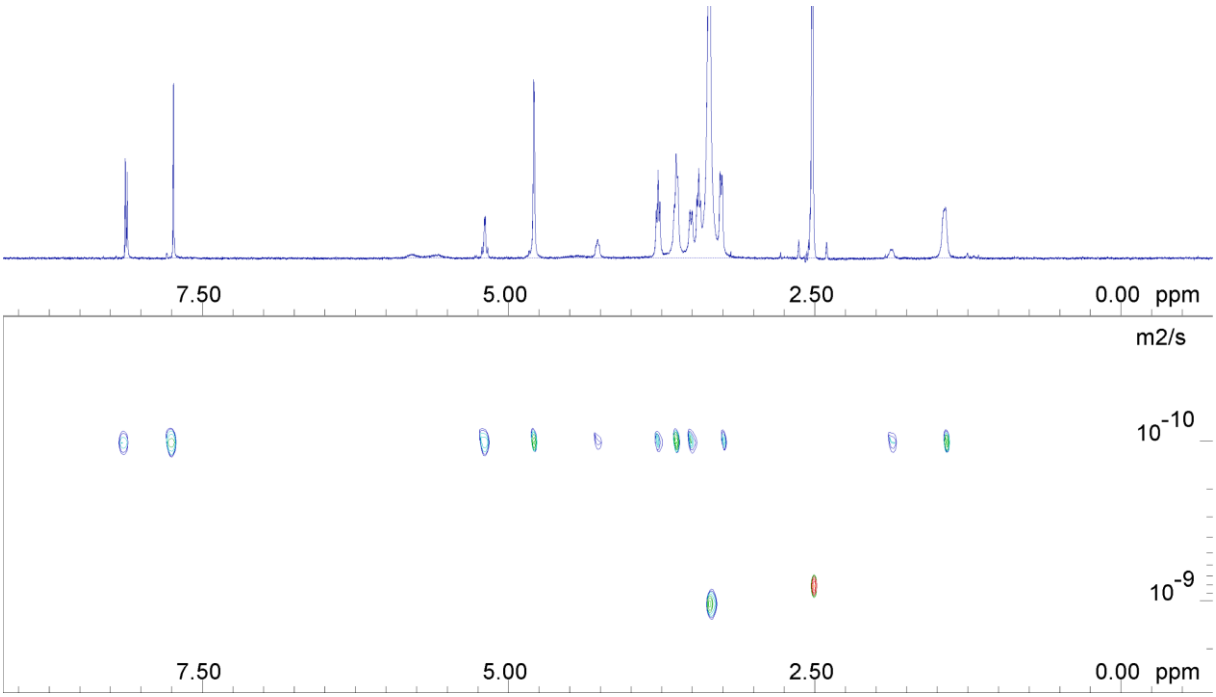


Figure S1. DOSY (600 MHz, D_2O) spectrum of CD [3]rotaxane **6**.

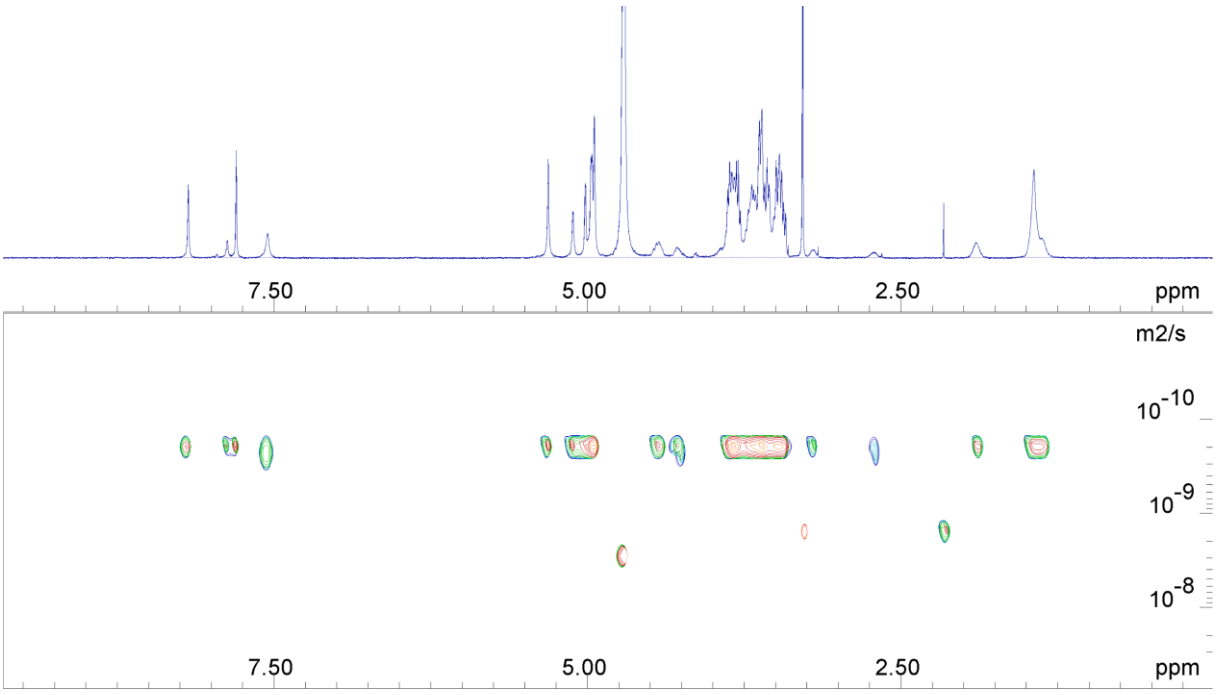


Figure S2. DOSY (600 MHz, D_2O) spectrum of **RotLa$_2$ 1a**.

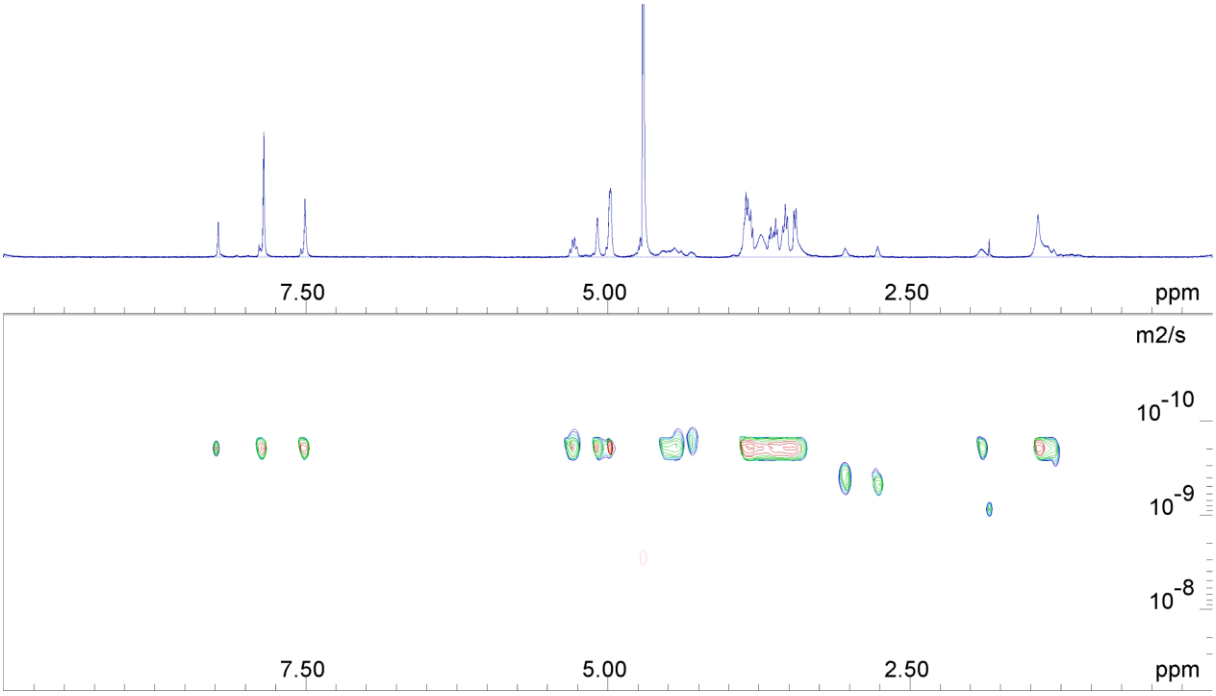


Figure S3DOSY (600 MHz, D_2O) spectrum of **RotLa$_4$ 2a**.

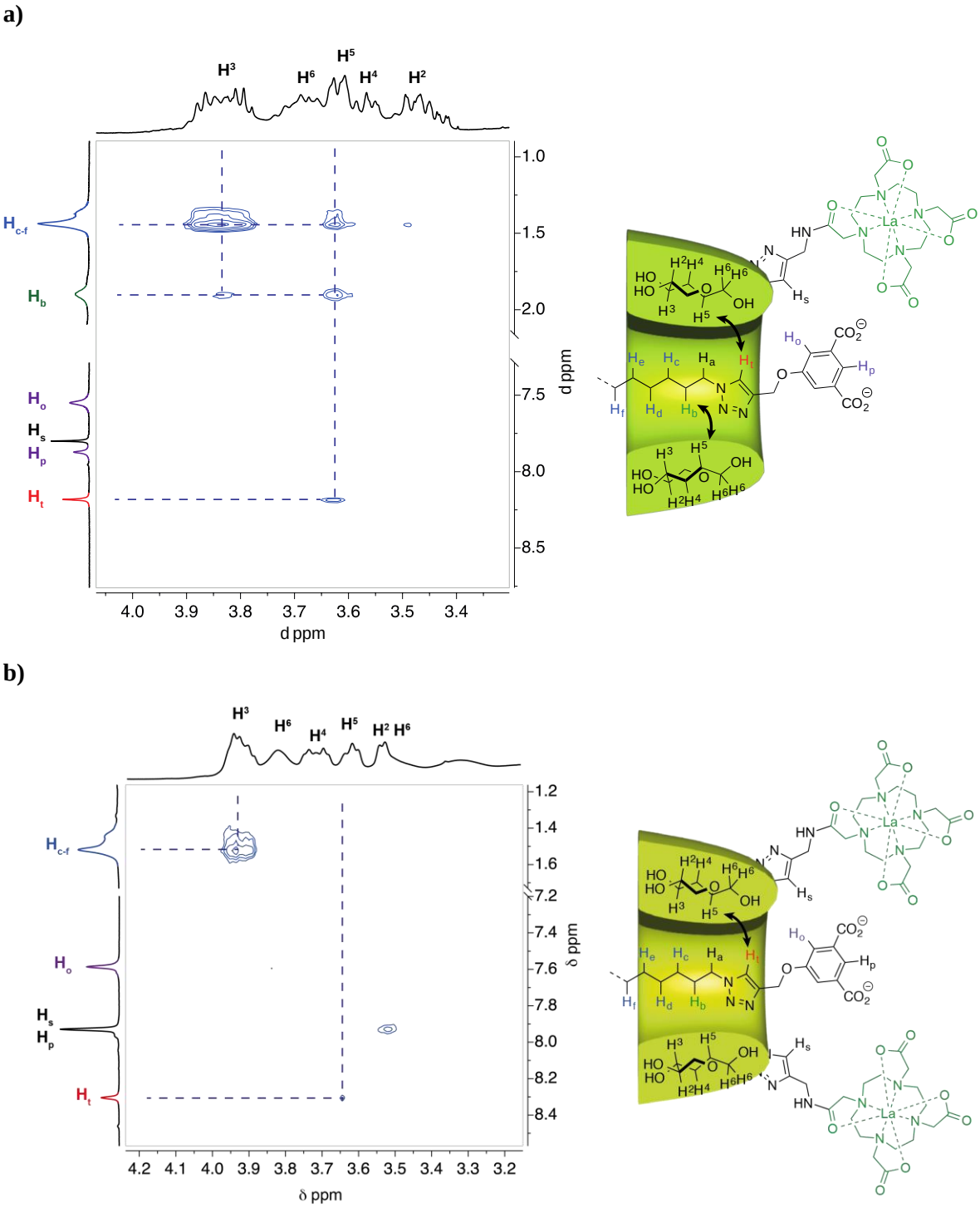
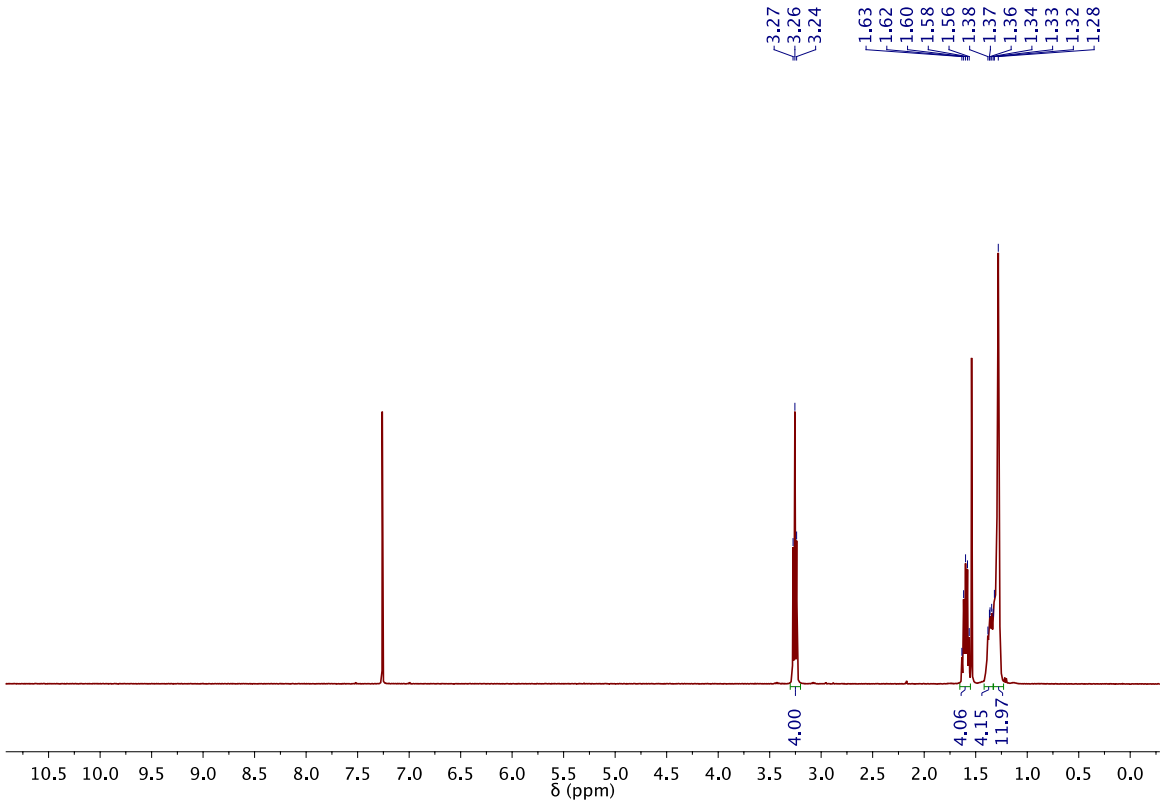


Figure S4.T-ROESY NMR of a) **RotLa$_2$1a**and b)**RotLa$_4$ 2a** (600 MHz, D_2O).

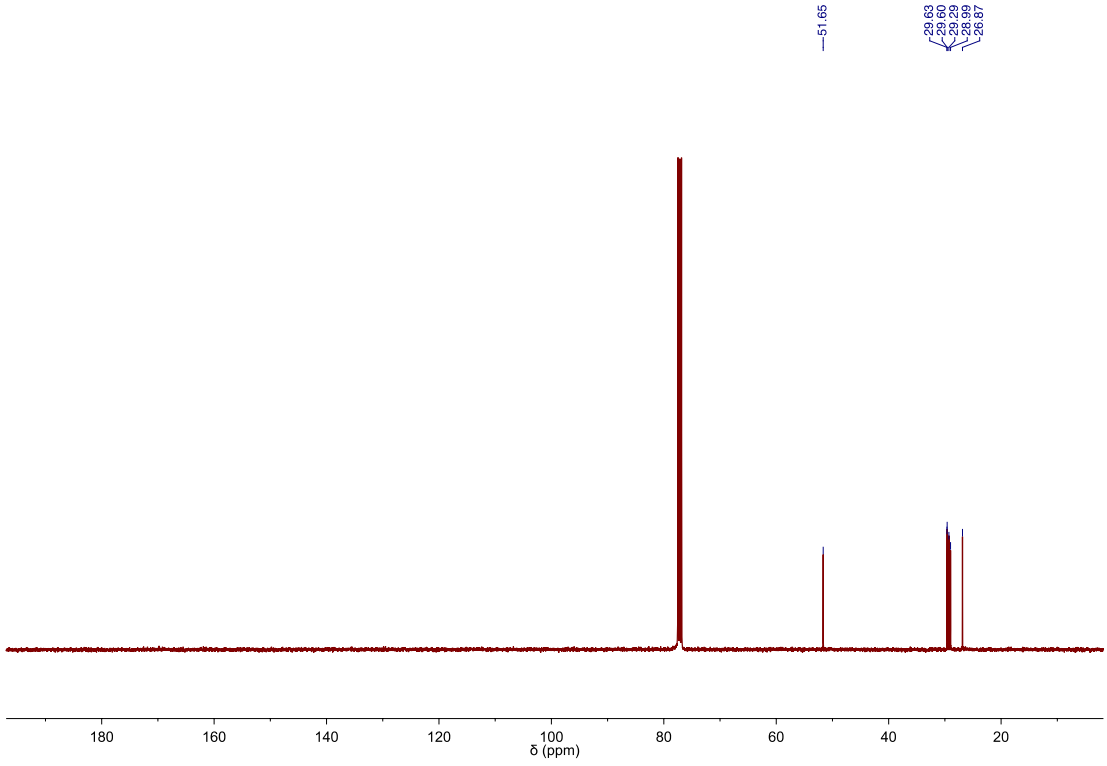
NMR and IR spectra of compounds

1,12-diazidododecane (**3**)

^{1}H NMR Spectrum

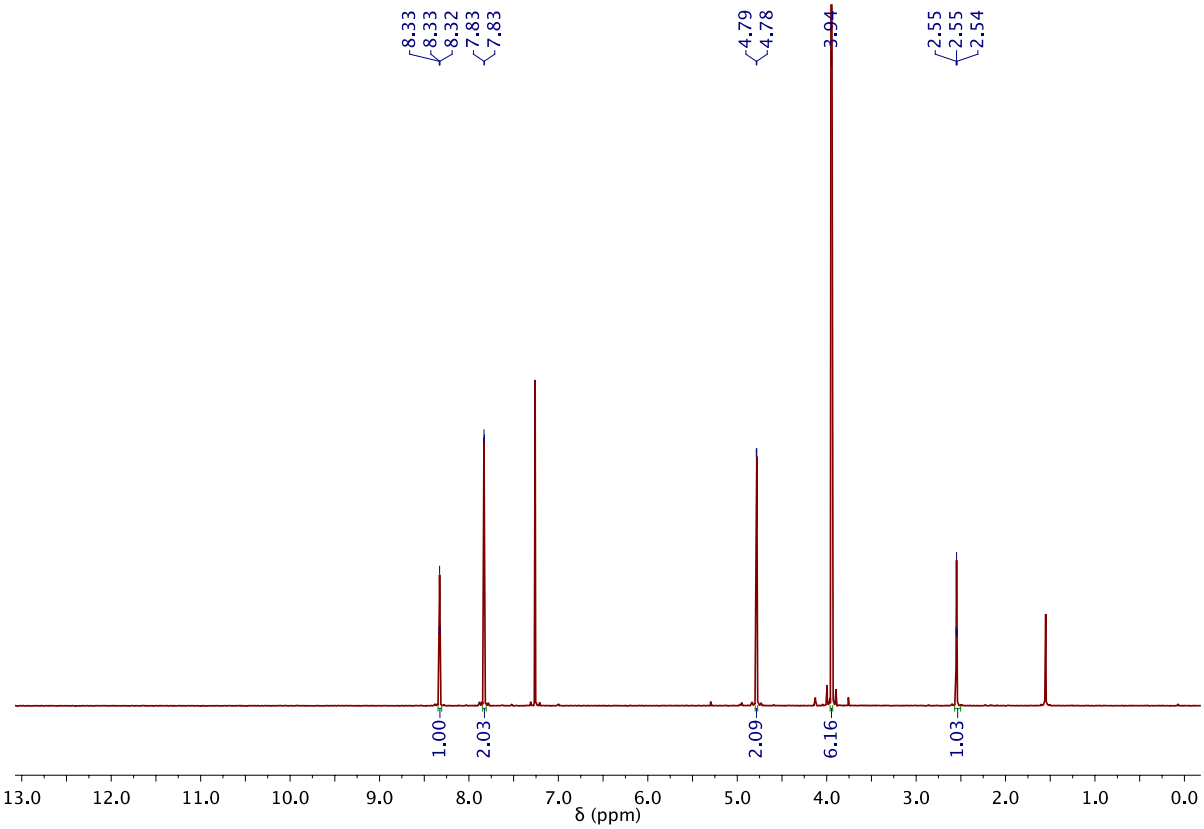


^{13}C NMR Spectrum



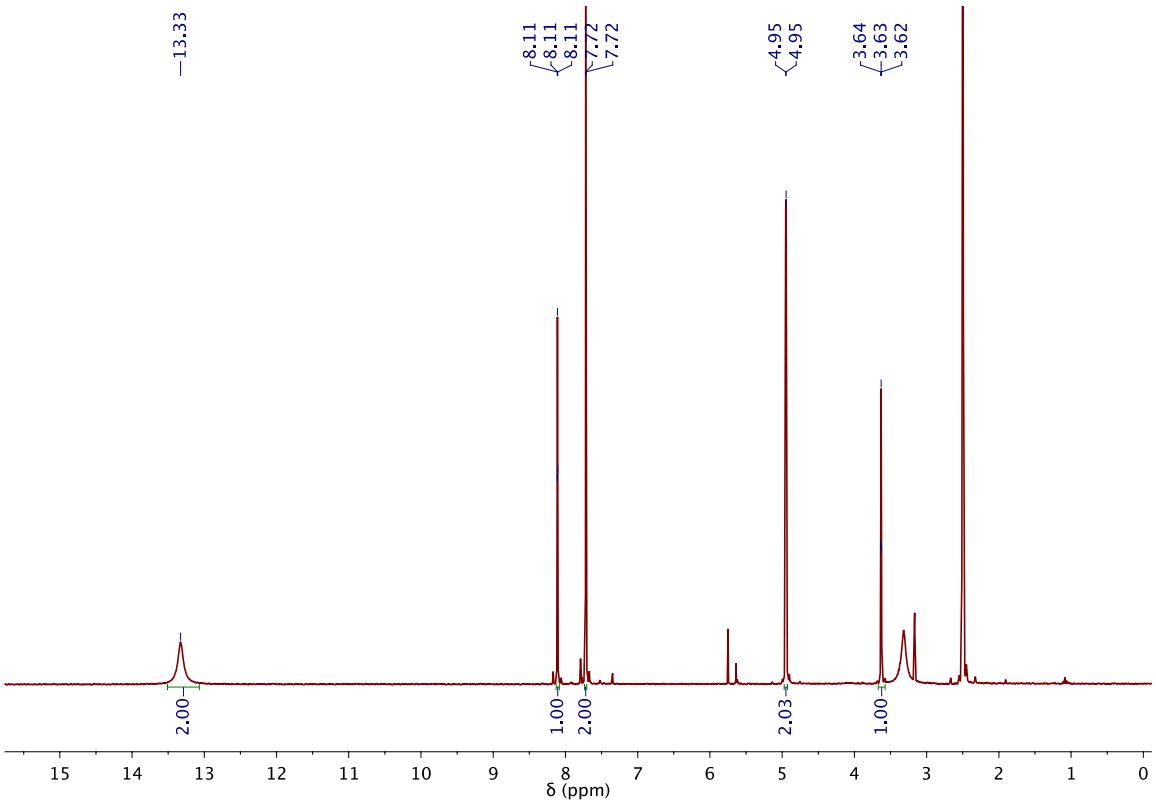
Dimethyl 5-(propargyloxy)isophthalate (**4**)

^{1}H NMR Spectrum

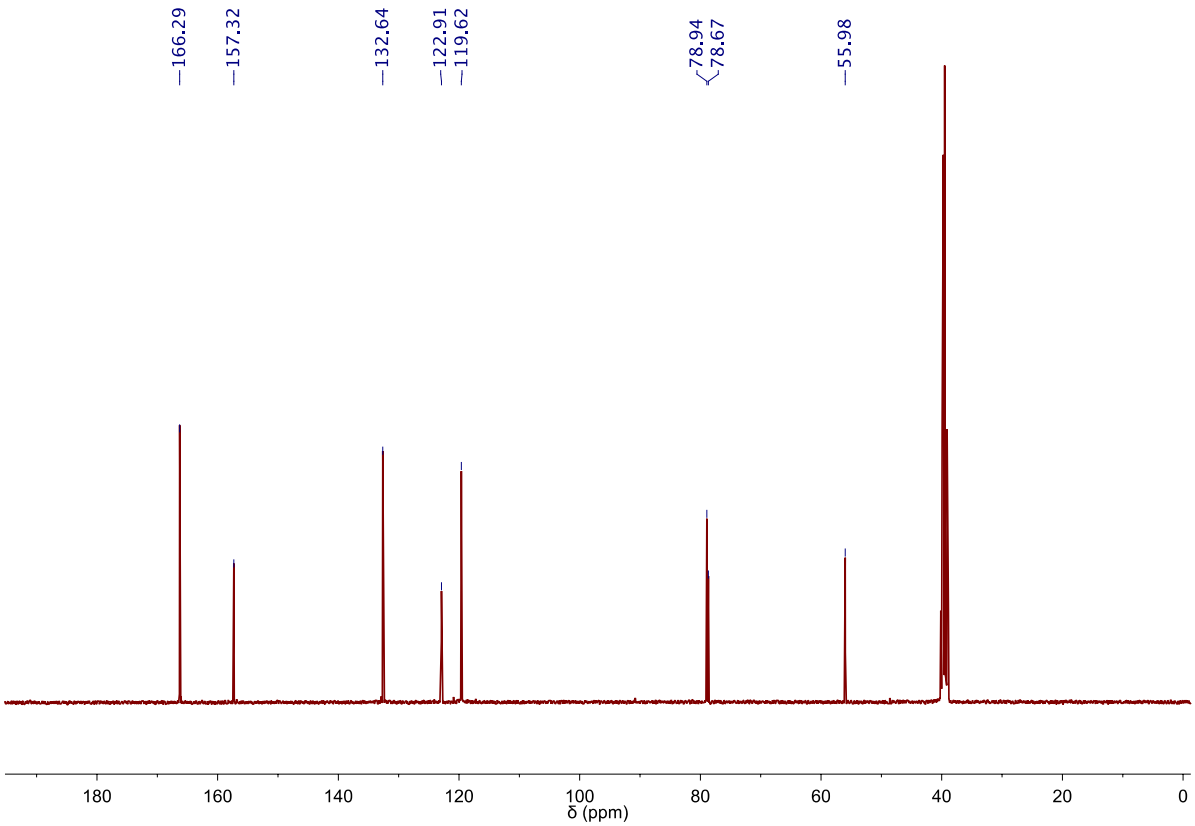


5-(propargyloxy)isophthalic acid (**5**)

^{1}H NMR Spectrum

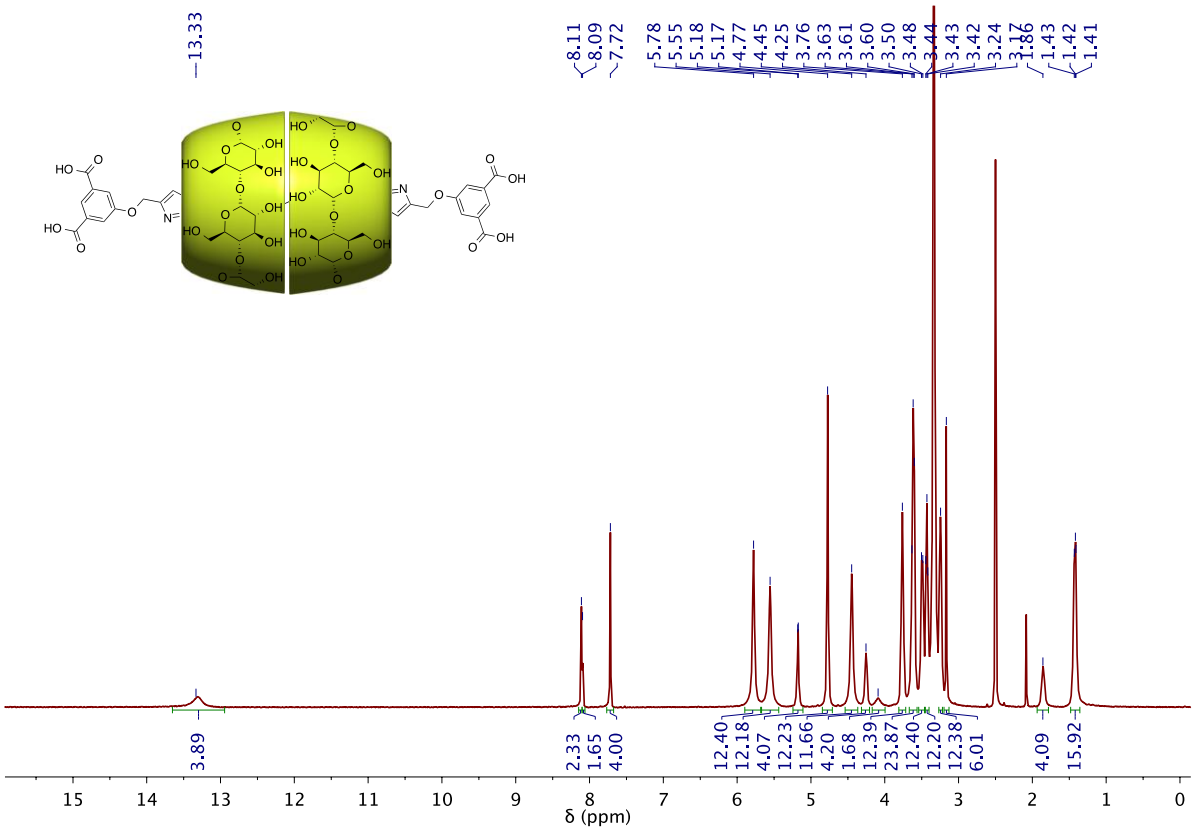


^{13}C NMR Spectrum

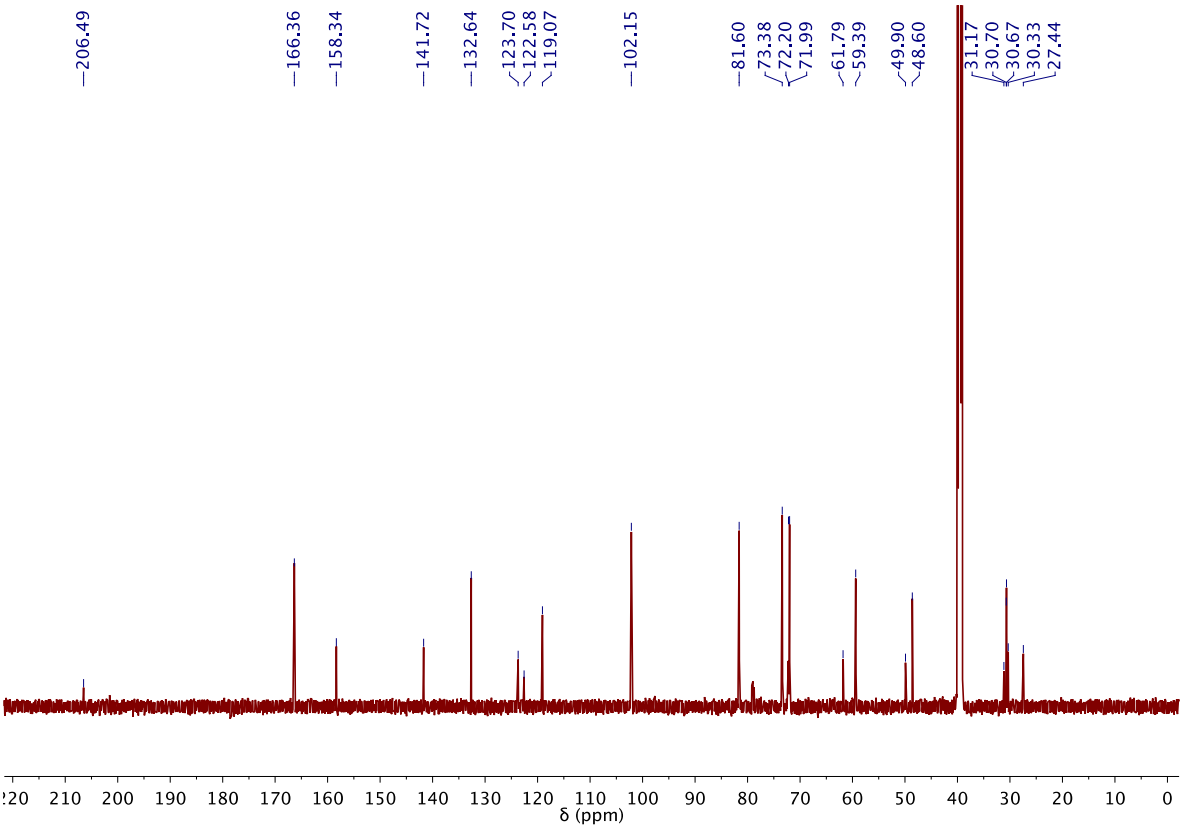


CD [3]rotaxane (**6**)

^{1}H NMR Spectrum



^{13}C NMR Spectrum



IR spectrum

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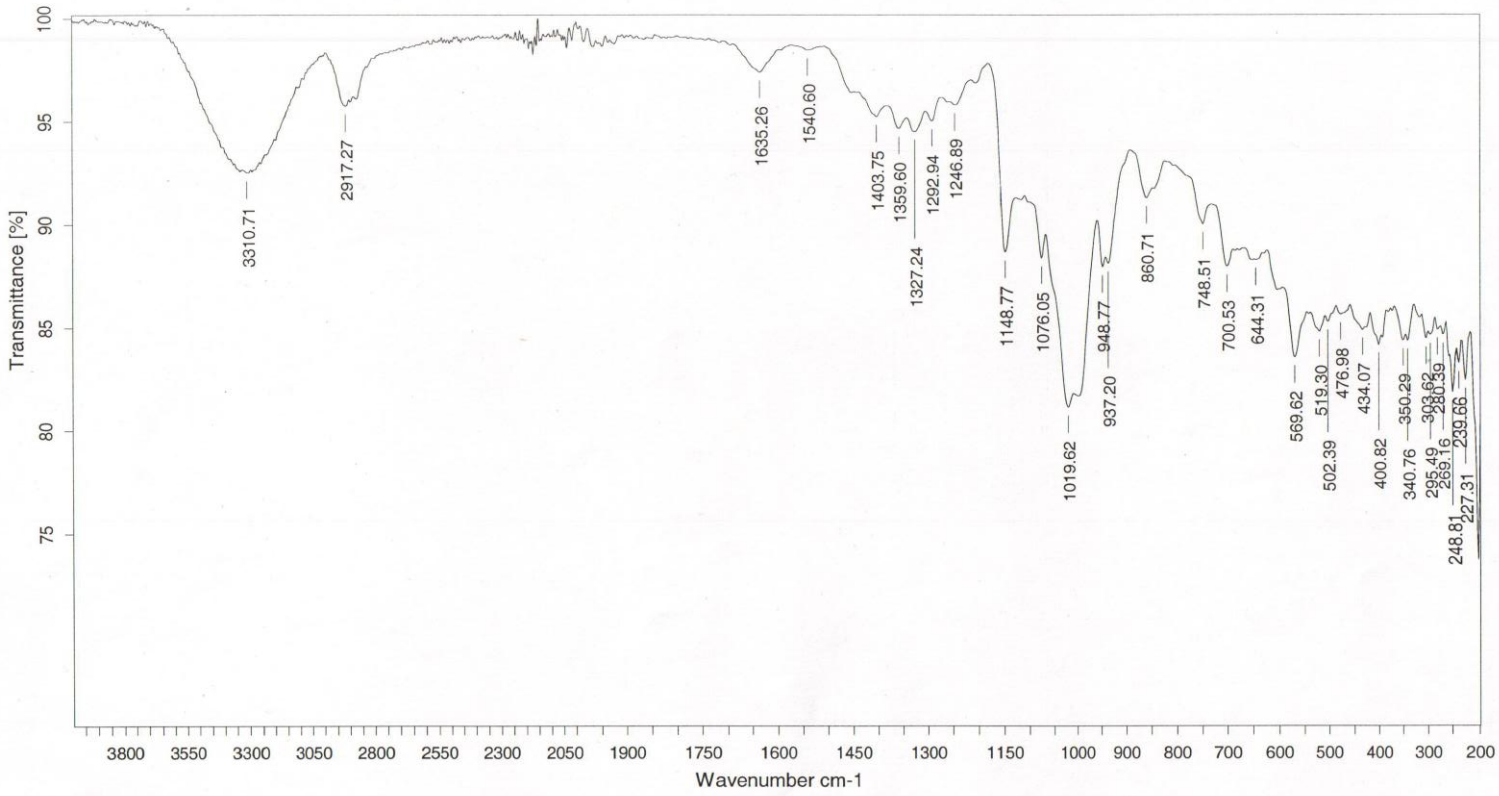


Echantillon : **3rotCD**

mesuré le 03/02/2017 sur TENSOR 27

résolution : 4 cm-1 (32 scans)

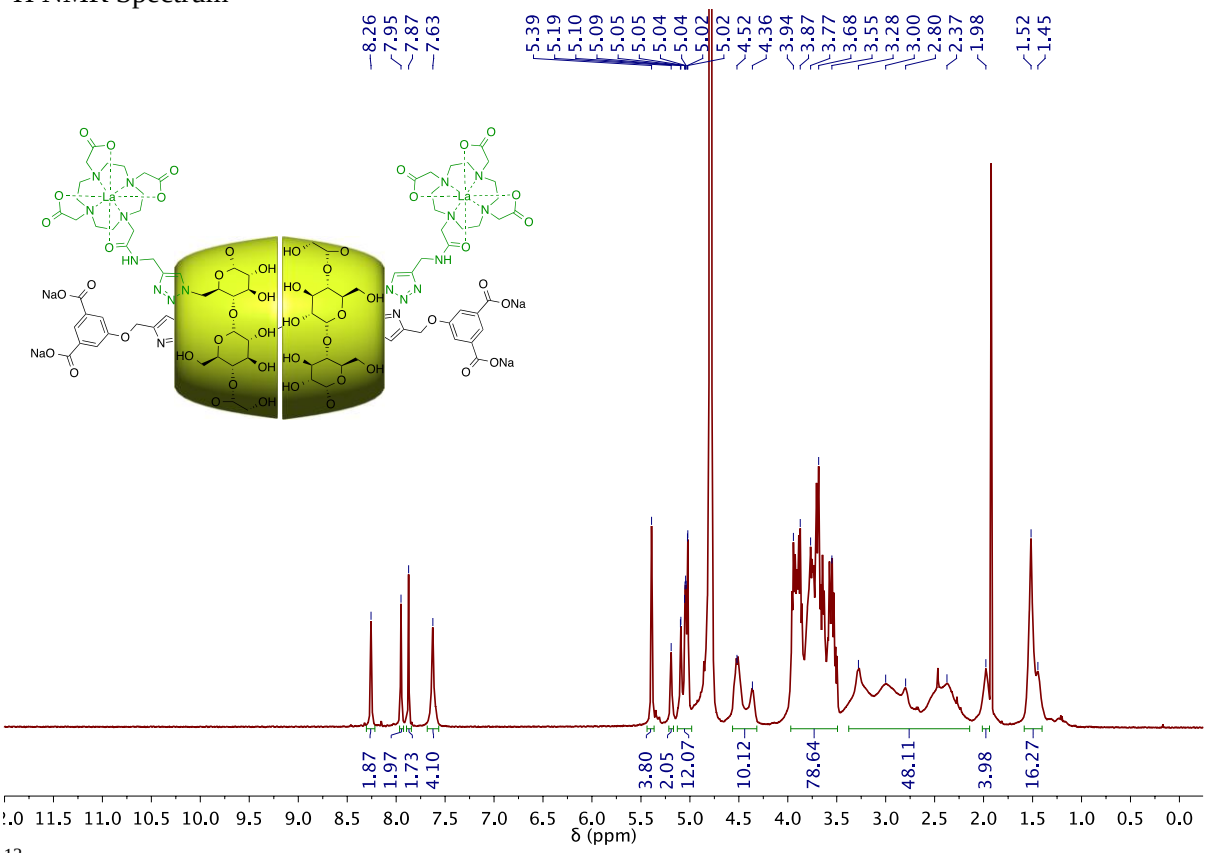
Spectre : 3rotCD.0 (dans C:\SPECTRES\SUPRA)

Technique : ATR

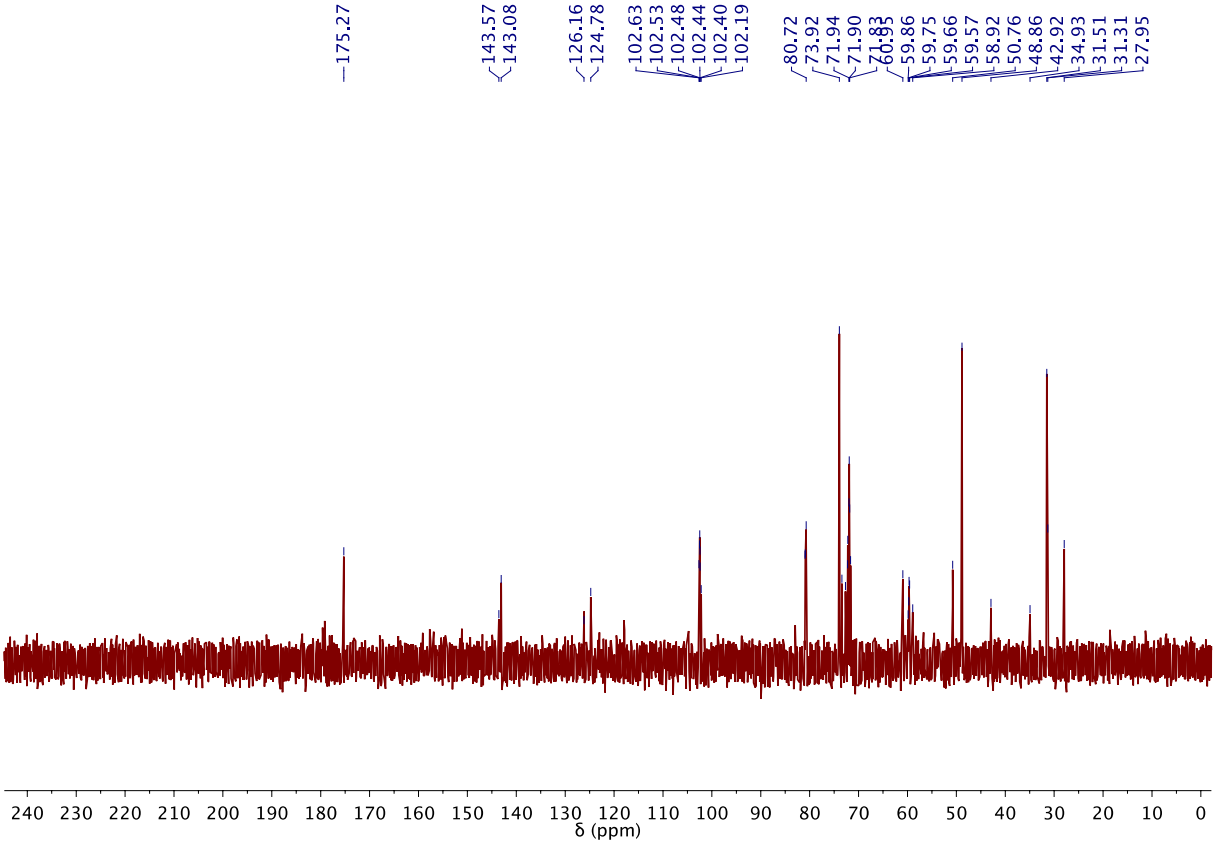
Opérateur : Guillaume

RotLa$_2$ (**1a**)

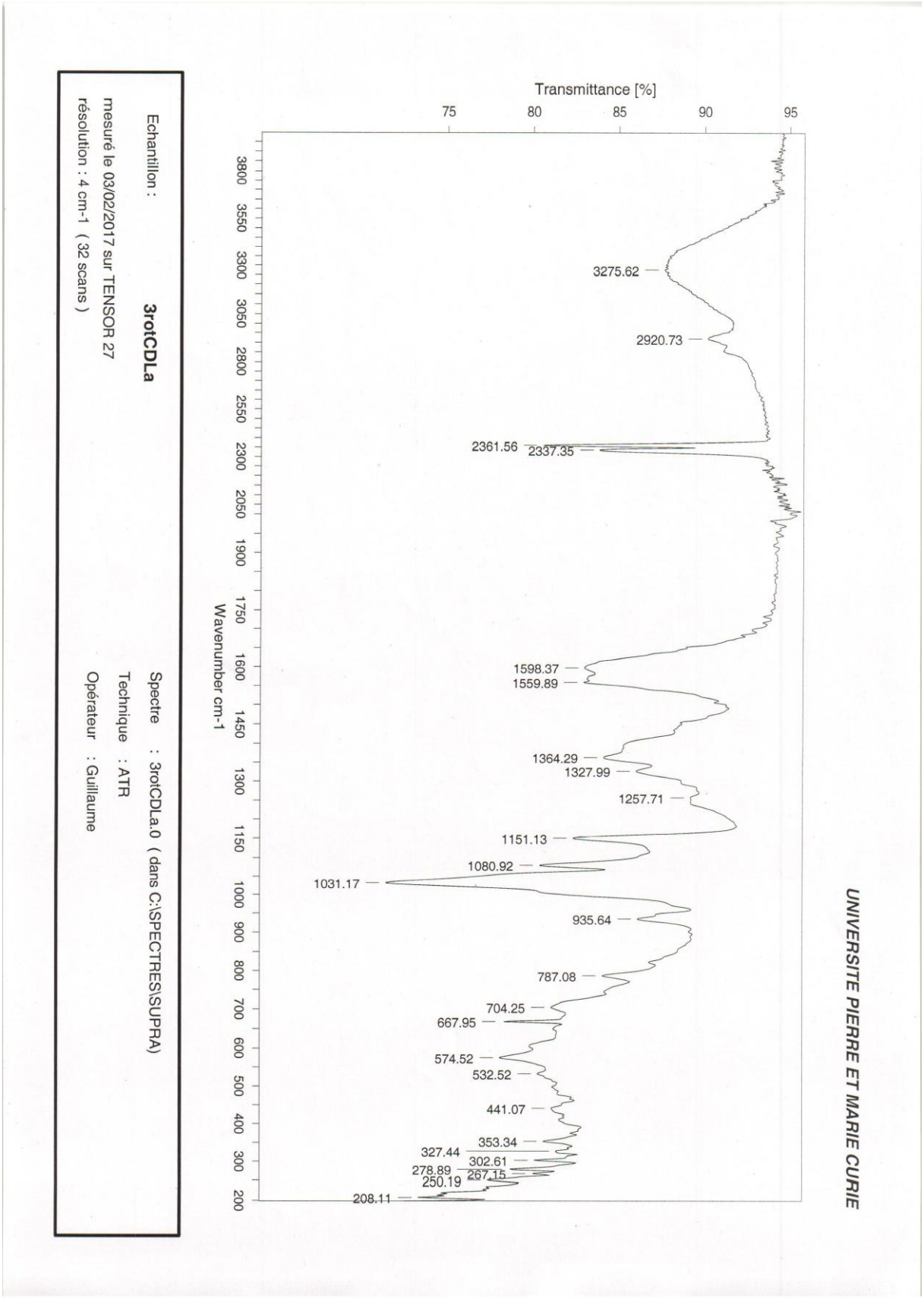
^{1}H NMR Spectrum



^{13}C NMR Spectrum



IR spectrum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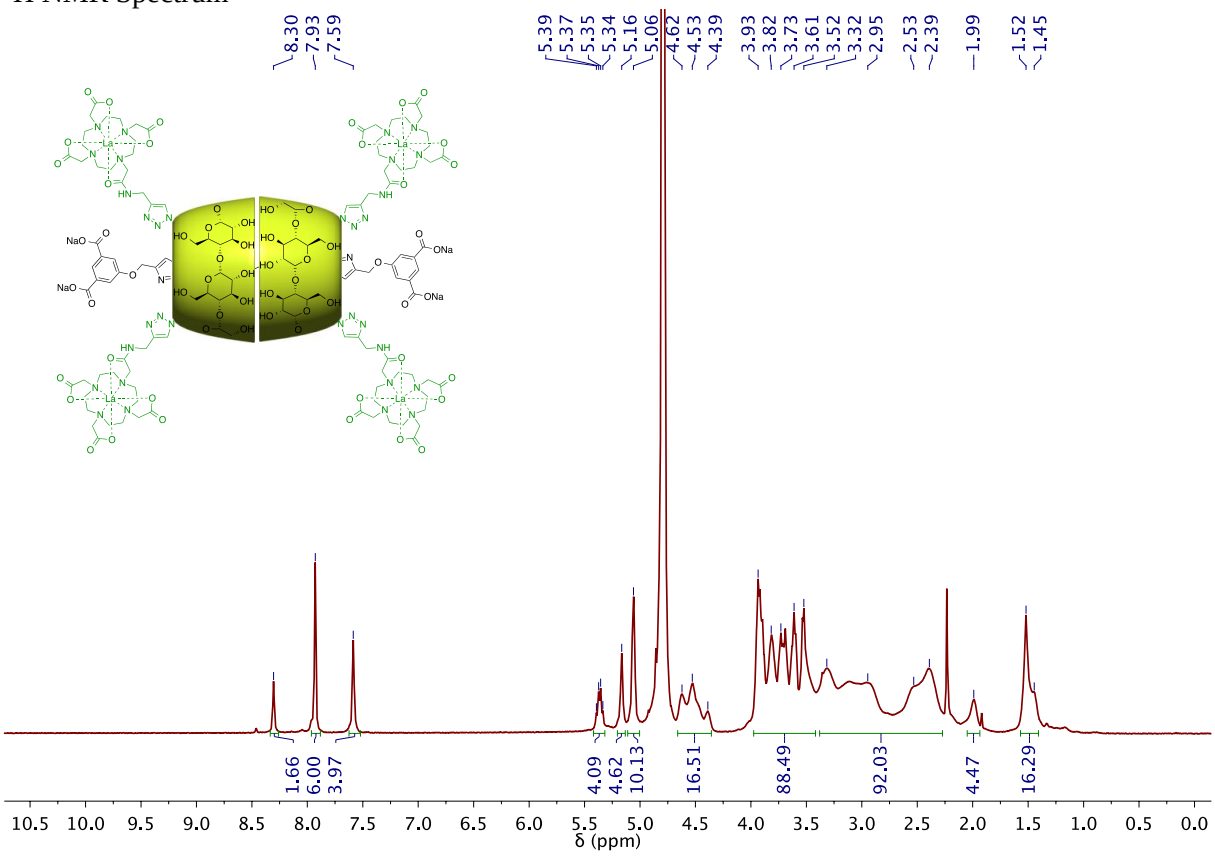
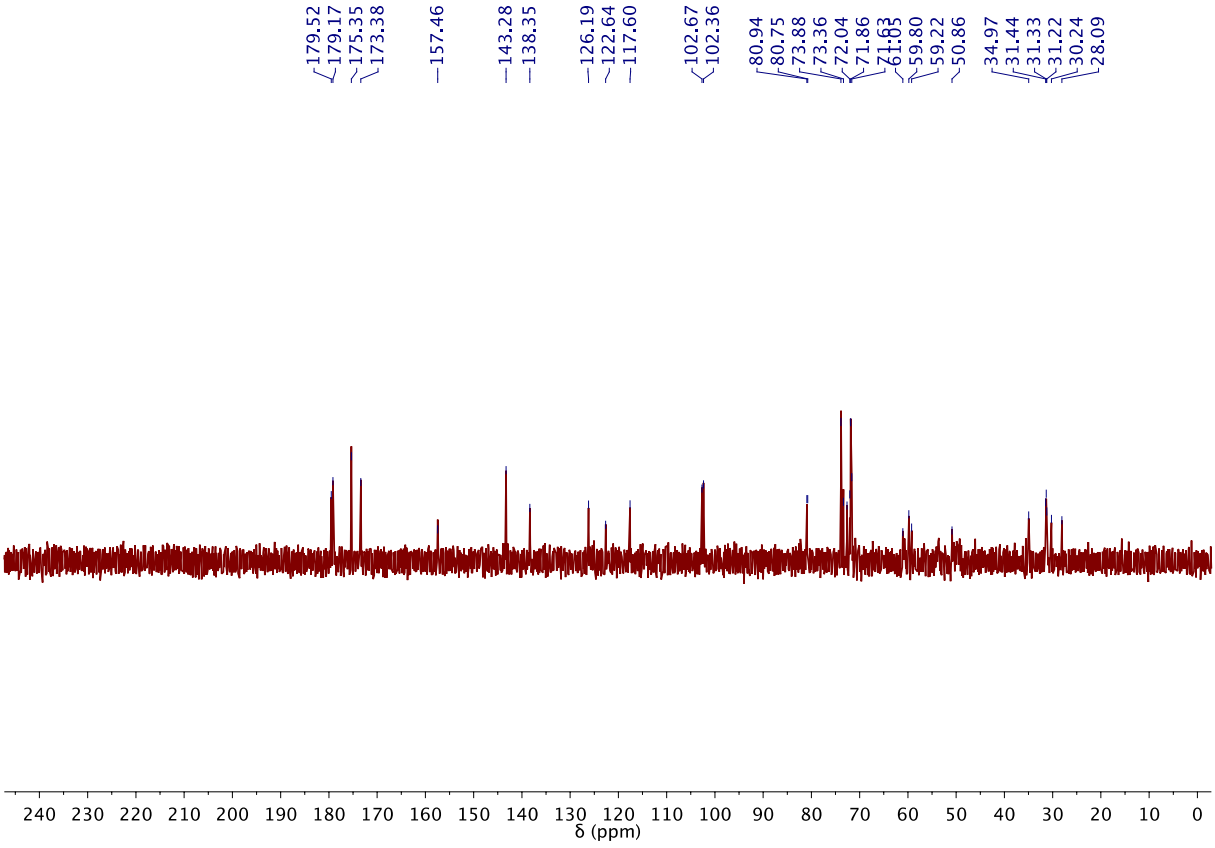
Transmittance [%]
75
80
85
90
95
3275.62
2920.73
2361.56
2337.35
1598.37
1559.89
1364.29
1327.99
1257.71
1151.13
1080.92
1031.17
935.64
787.08
704.25
667.95
574.52
532.52
441.07
353.34
327.44
302.61
278.89
267.15
250.19
208.11
Wavenumber cm-1
3800 3550 3300 3050 2800 2550 2300 2050 1900 1750 1600 1450 1300 1150 1000 900 800 700 600 500 400 300 200
Echantillon : 3rotCDLa
mesuré le 03/02/2017 sur TENSOR 27
résolution : 4 cm-1 (32 scans)
Spectre : 3rotCDLa.0 (dans C:\SPECTRES\SUPRA)
Technique : ATR
Opérateur : Guillaume

RotLa$_4$ (**2a**)

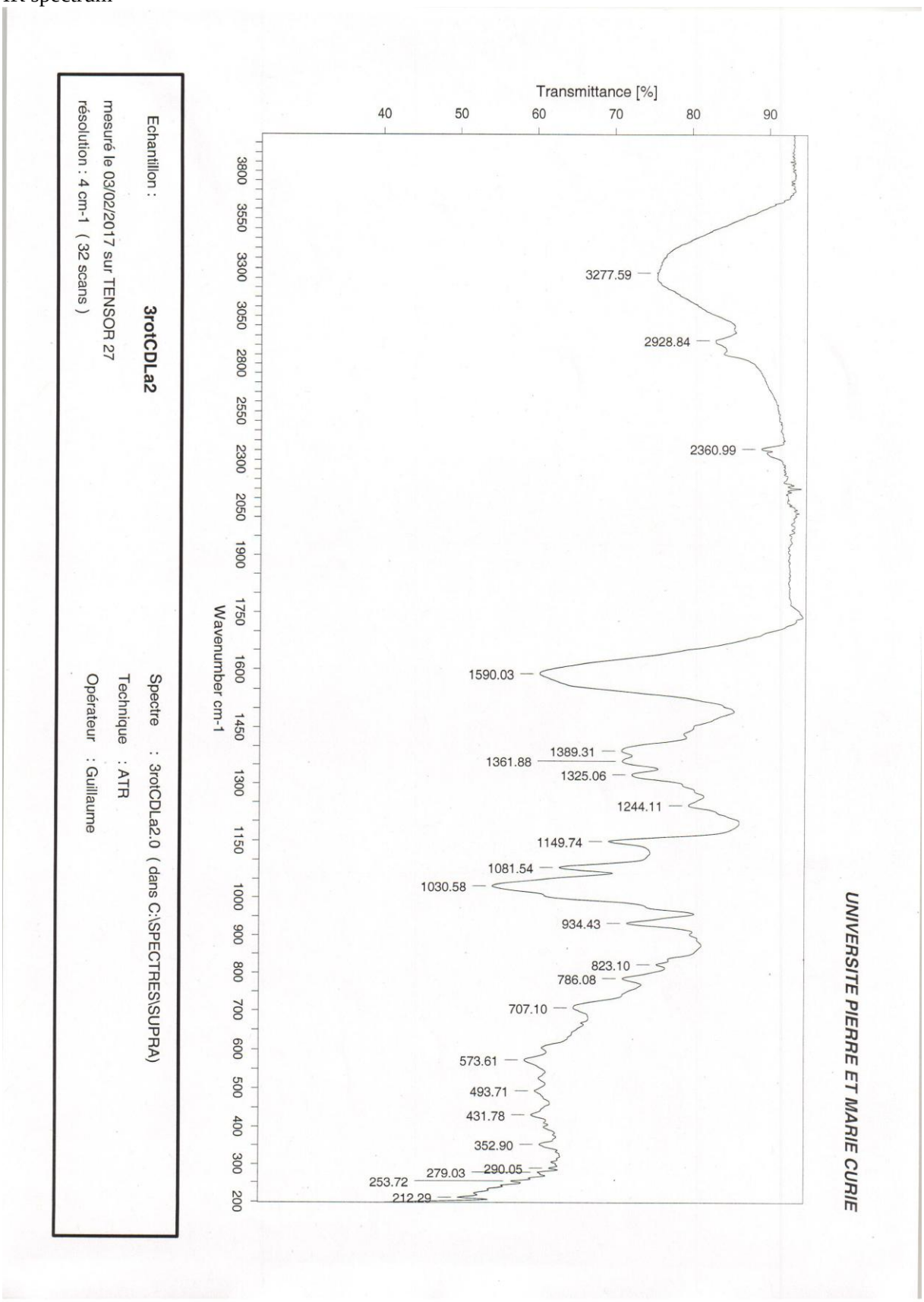
^{1}H NMR Spectrum



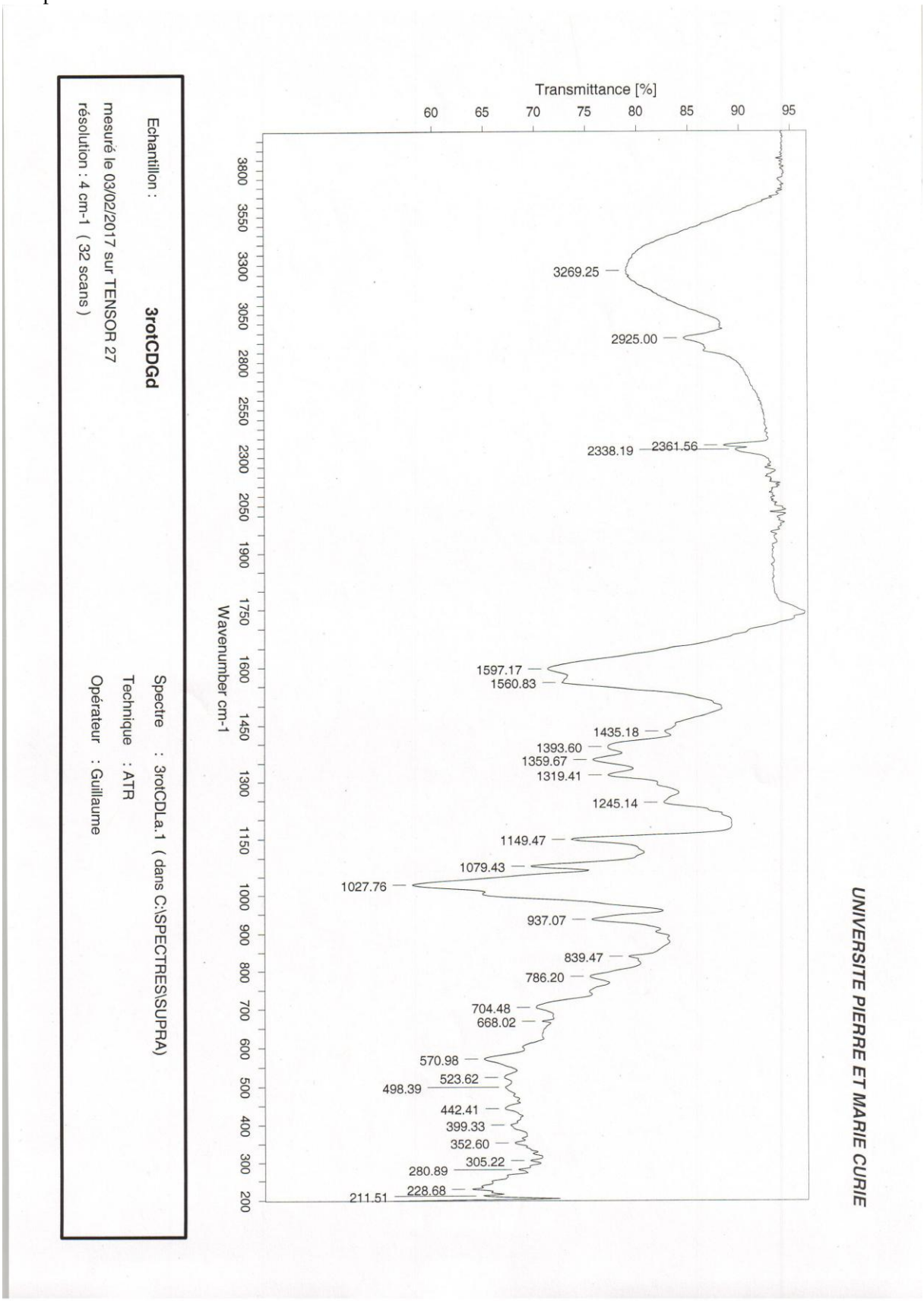
^{13}C NMR Spectrum



IR spectrum


Transmittance [%]
40
50
60
70
80
90
Wavenumber cm-1
3800
3550
3300
3050
2800
2550
2300
2050
1900
1750
1600
1450
1300
1150
1000
900
800
700
600
500
400
300
200
3277.59
2928.84
2360.99
1590.03
1389.31
1361.88
1325.06
1244.11
1149.74
1081.54
1030.58
934.43
823.10
786.08
707.10
573.61
493.71
431.78
352.90
290.05
279.03
253.72
212.29
UNIVERSITE PIERRE ET MARIE CURIE
Echantillon : 3rotCDLa2
mesuré le 03/02/2017 sur TENSOR 27
résolution : 4 cm-1 (32 scans)
Spectre : 3rotCDLa2.0 (dans C:\SPECTRES\SUPRA)
Technique : ATR
Opérateur : Guillaume

IR Spectrum of **1b**



Mass spectra of rotaxanes

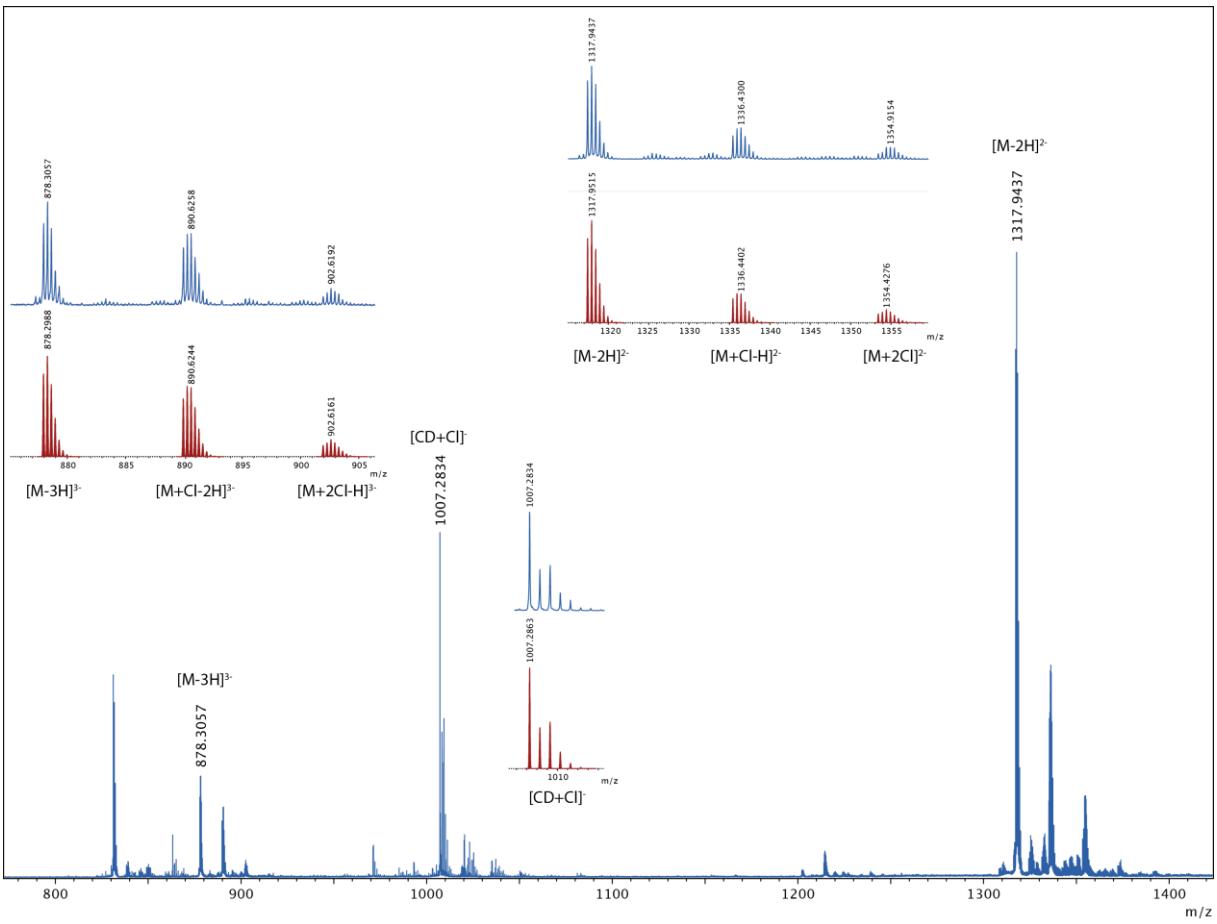


Figure S5HRMS (ESI) spectrum of CD [3]rotaxane**6**

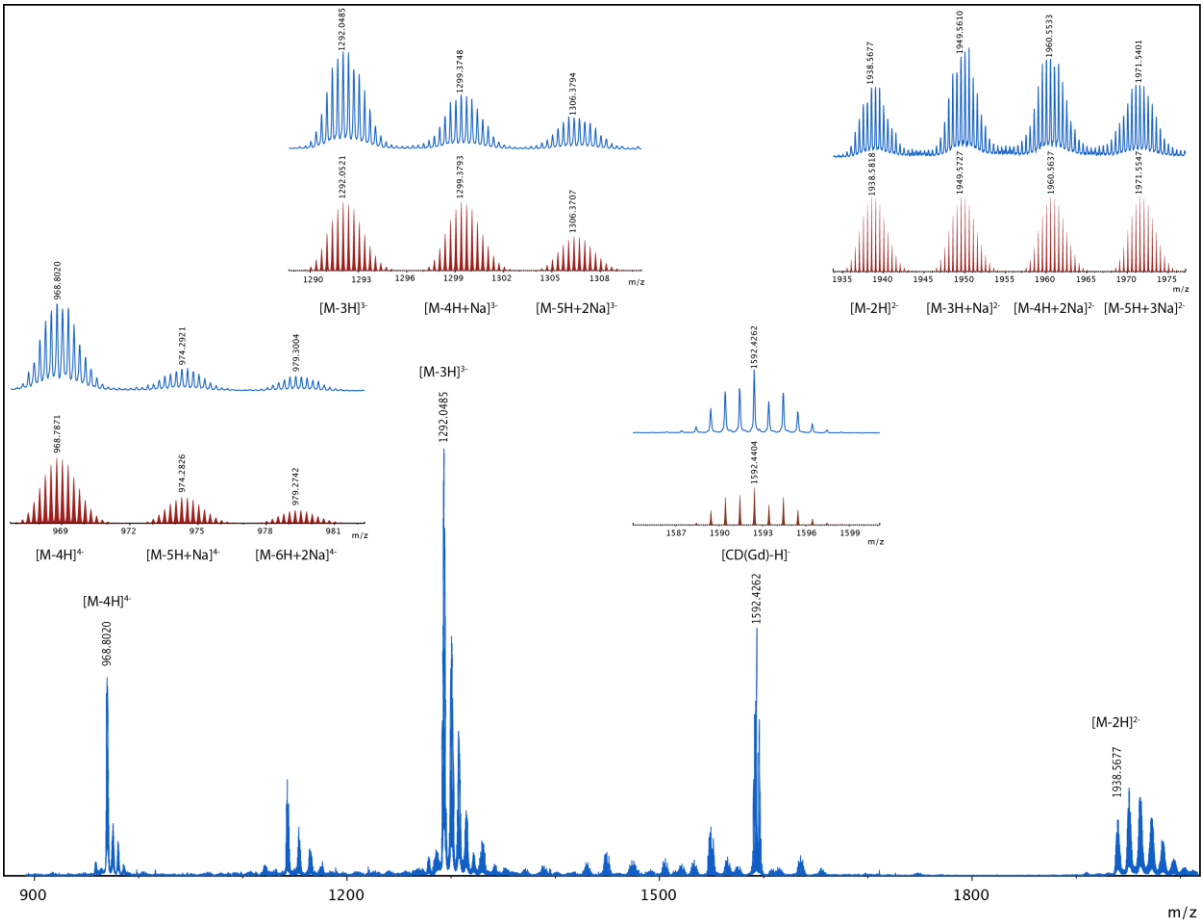


Figure S6 HRMS (ESI) spectrum of **RotGd$_2$ 1b**.

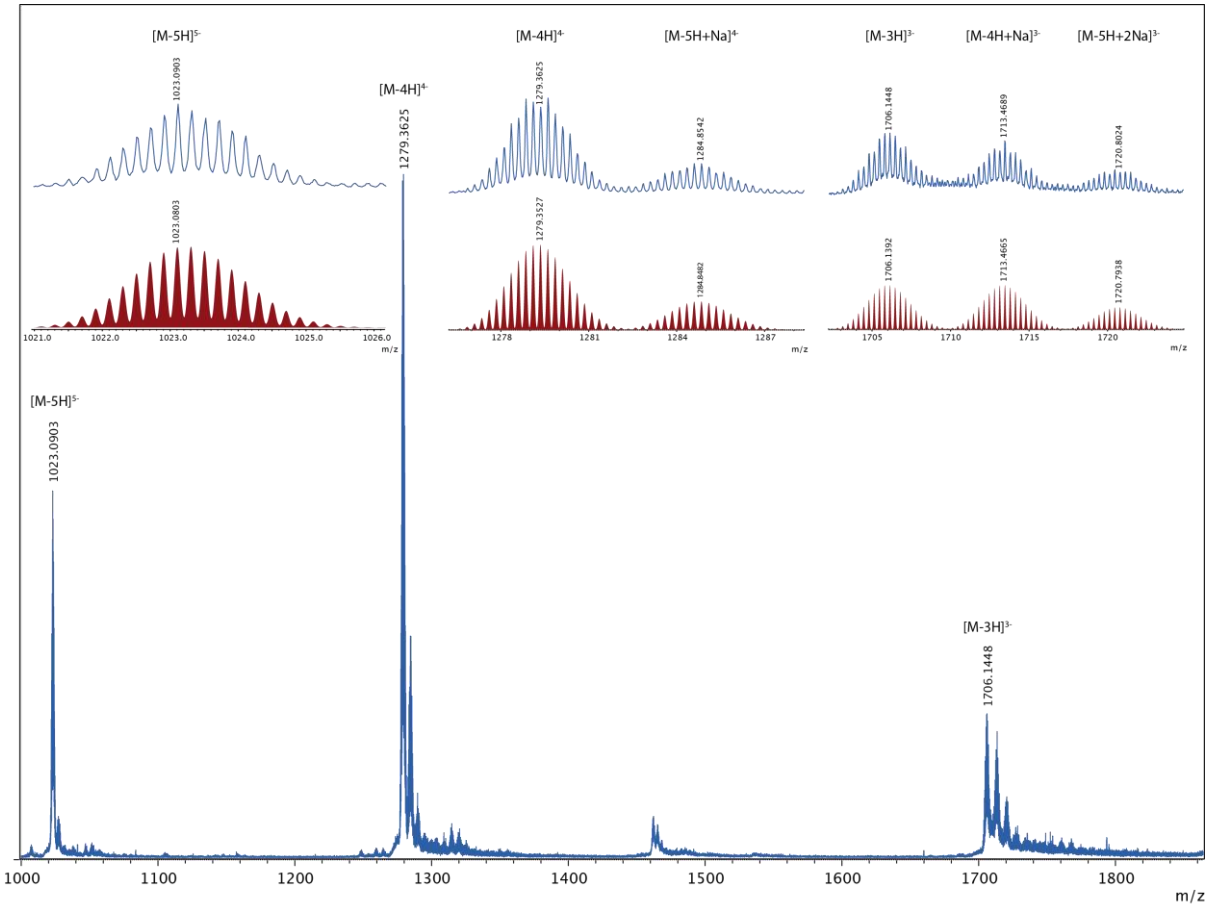


Figure S7HRMS (ESI) spectrum of **RotGd$_4$ 2b**.

Relaxivities

Table S1. Relaxivities ($mM^{-1} \cdot s^{-1}$) of functionalized CD and [3]rotaxanes at 25°C and 37°C.

	r_1 at 20 MHz		**r_1 at 60 MHz**		**r_1 at 300 MHz**
	25°C	37°C	25°C	37°C	25°C
Gd-DOTA		3.83		3.0	
CDGd 7b	7.87	7.06	7.56	6.70	5.83
$CDGd_2$ 8b	9.78	8.57	8.99	8.33	6.27
$RotGd_2$ 1b	11.90	12.30	11.47	11.98	5.85
$RotGd_4$ 2b	14.29	15.70	13.60	15.00	6.18

MTT assay

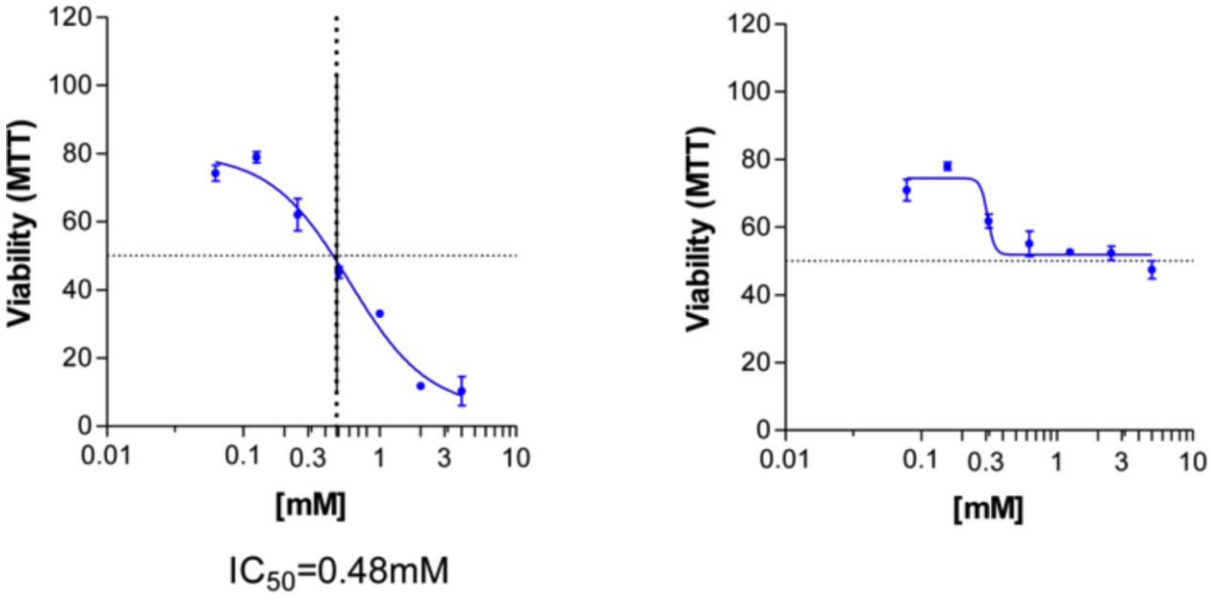


Figure S8 MTT cytotoxicity assay of**RotGd$_2$**(left) and **RotGd$_4$**(right). The cell lines used are hepatocytes BWGT3 (from ATCC). The exposure time was 1h.

MRI Cross-sections

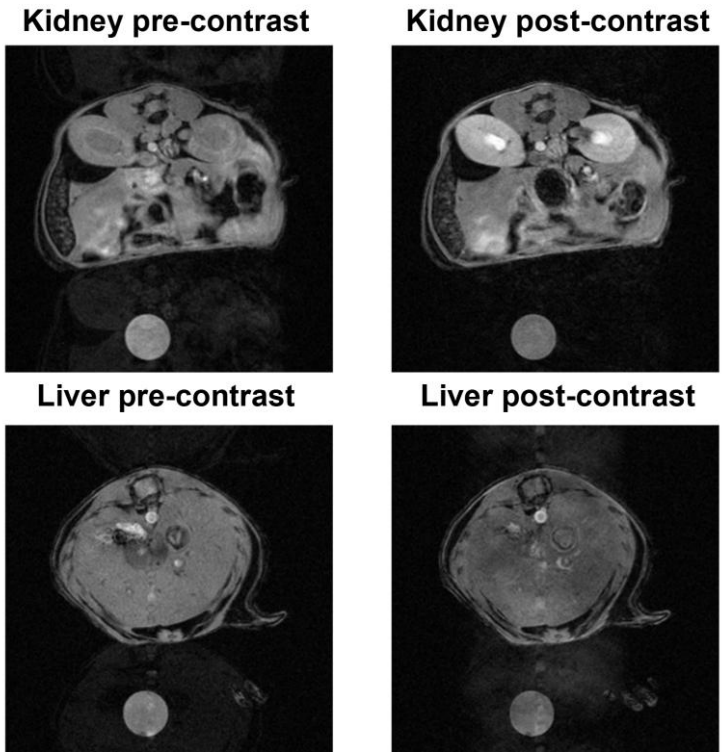


Figure S9MRI cross-sections of the kidney pre and post-injection of **RotGd$_2$**

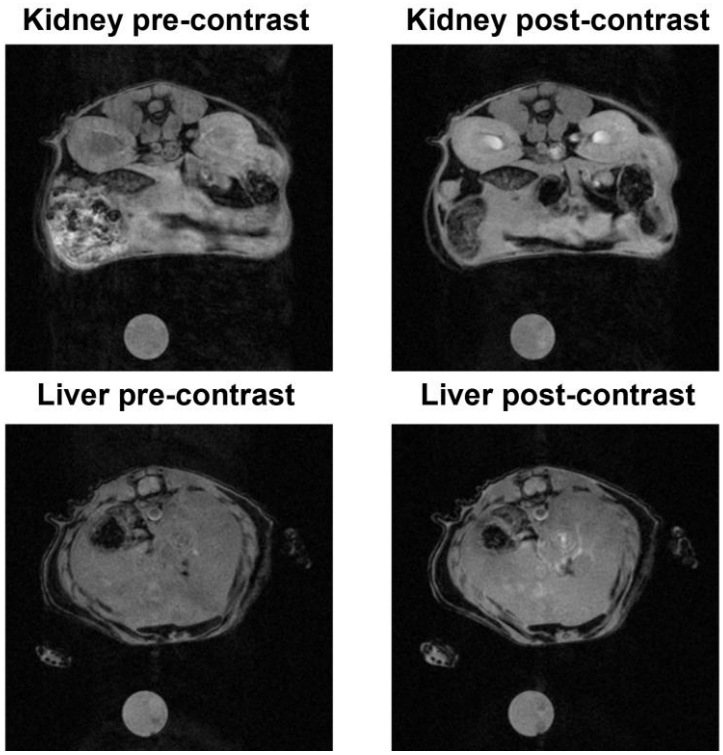


Figure S10MRI cross-sections of the kidney pre and post-injection of **RotGd$_4$**

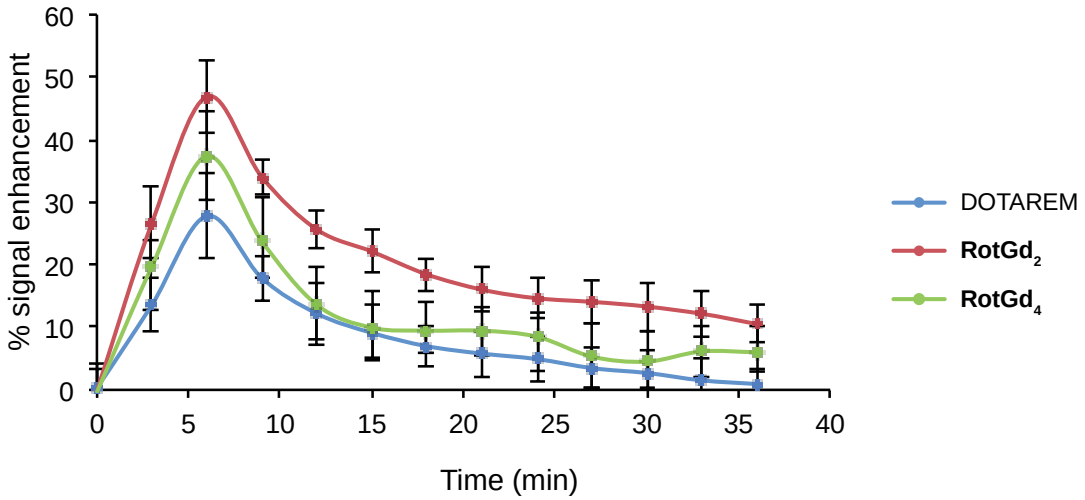


Figure S11Dynamic MRI signal enhancement in the liver after CA injection.

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[2] R. Rajaganesh, J. Jayakumar, C. Sivaraj, N. Raaman, T. M. Das, *Carbohydr. Res.* **2010**, *345*, 1649-1657.

[3] Y.-L. Zhao, W. R. Dichtel, A. Trabolsi, S. Saha, I. Aprahamian, J. F. Stoddart, *J. Am. Chem. Soc.* **2008**, *130*, 11294-11296.