Selective synthesis of an elusive *C*-functionalized bis-cyclam and study of its inhibition properties of CXCR4 chemokine receptor

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SUPPORTING INFORMATION

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Experimental details

Synthesis. Reagent used for synthesis were purchased from SIGMA-ALDRICH®, TCI chemicals®, ACROS ORGANICS® and Ambeed® and used without further purification. Solvents for synthesis were obtained from a MBraun MB-SPS 800 purification system. Ultrapure water was freshly obtained from a Milli-Q dispenser.

Compounds **3** and **8** were synthesized according to previously reported procedures. [38,32]

Nuclear Magnetic Resonance Spectroscopy. NMR data were recorded at the "Service Général des Plateformes (SGPLAT)" of the Université de Bretagne Occidentale. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker Avance III HD 500 (500.25 MHz for ^{1}H and 125.79 MHz for ^{13}C), Bruker Avance 400 (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) or Bruker AMX-3 300 (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C) spectrometers. Deuterated solvents from Eurisotop® are used to reference spectra, ^{1}H and ^{13}C shifts are reported in ppm and the δ scales are relative to TMS.

The signals are indicated as follows: chemical shift, multiplicity (s for singlet; br for broad singlet, d for doublet; t for triplet; q for quadruplet; m for multiplet), coupling constants J in Hertz (Hz), assignment: $CH_2\alpha N$, $CH_2\beta N$ or $CH_2\gamma N$ correspond to CH_2 located in alpha, beta or gamma position of considered nitrogen atom, type of nuclei is indicated in italic. Ar is a generic term used in subscript for all H or C aromatic atoms.

Mass Spectrometry. High-Resolution Mass Spectrometry (HRMS) analyses were performed at the Institute of Organic and Analytic Chemistry (ICOA Orléans) on a HRMS Q-Tof MaXis, sources ESI, APCI, APPI and nano-ESI. MALDI TOF were recorded at the "service commun" of the Université de Bretagne Occidentale using MALDI TOF-TOF AuTOFLEX III from Bruker Daltonics.

Liquid Chromatography (HPLC). HPLC analysis was carried out on Shimadzu LD20 system equipped with an Agilent Zorbax SB-C18 column (4.6 x 250 mm, 5-micron) and a Shimadzu ELSD LTII detector (80°C, N_2 as nebulizing gas, 350kPa). Elution with H_2O (0.1% formic acid)/Acetonitrile was performed with 30 minutes program set up as: 95:5 for 16 mins, gradient to 10:90 in 6 mins + 2 mins at 10:90, gradient to 95:5 in 6 mins). Analysis and data were processed with LabSolutions software.

Synthetic protocols and characterization data for compounds 1-10

Synthesis of 1: Isophtaldehyde (1.000 g, 7.46 mmol), Meldrum Acid (2.146 g, 14.9 mmol) and Hantzch ester (3.777 g, 14.9 mmol) were solubilized in MeOH (26 mL). Then, *L*-proline (0.172 g, 1.49 mmol, 20 %) was added and the reaction mixture was stirred at 25 °C for 12 h. The precipitate was filtered and washed with diethyl ether. The crude product was purified by column chromatography on silica gel (Hexane/Ethyl acetate/Methanol 50:50:0 to 0:90:10) to afford compound **1** as a white powder (2.460 g, 85 %).

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K) δ (ppm) 7.24-7.17 (m, 4H, CH_{Ar}), 3.77 (t, 2H, C*H*, J = 5.21 Hz), 3.44 (d, 4H, CH_2, J = 5.21 Hz), 1.52 (s, 6H, CH_3), 1.55 (s, 6H, CH_3). *^{13}C Jmod NMR* ($CDCl_3$, 75 MHz, 298 K) δ (ppm) 165.3 (*C*O), 137.8 (C_{ipso}), [130.7, 129.0, 128.6 (x2)] (*C*H_{Ar}), 105.4 (C_{sp}), 48.2 (*C*H), 31.9 (*C*H_2), [28.6, 27.2] (*C*H_3). *HRMS* (ESI, positive, H_2O) m/z calcd. for $[C_{20}H_{26}NO_8]^+$ 408.1653 found $[M+NH_4]^+$ 408.1651, calcd. for $[C_{20}H_{22}NaO_8]^+$ 413.1207 found $[M+Na]^+$ 413.1207. *Mp*: 180 °C

Synthesis of 2: Compound **1** (2.460 g, 6.30 mmol) and Eschenmoser's salt (5.831 g, 31.5 mmol) were solubilized in MeOH (25 mL), and the reaction was stirred at 65 °C for 12 h. After cooling to room temperature, the solvent was removed under vacuum. The crude product was dissolved in $CHCl_3$ (100 mL) and washed with $NaHCO_{3(sat)}$ (50 mL), $KHSO_{4(10\ \%\ weight)}$ (50 mL) and $NaCl_{(sat)}$ (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford compound **2** as an orange oil (1.674 g, 97 %).

^{1}H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm) 7.22-7.19 (m, 1H, CH_{Ar}), 7.06-7.03 (m, 3H, CH_{Ar}), 6.22 (s, 2H, =C*H*), 5.43 (s, 2H, =C*H*), 3.73 (s, 6H, CH_3), 3.60 (s, 4H, CH_2). *^{13}C Jmod NMR* ($CDCl_3$, 75 MHz, 298 K) δ (ppm) 167.3 (*C*O), [140.1, 138.8] (C_{ipso}), [129.8, 128.5, 127.1 (x2)] (*C*H_{Ar}), 126.2 (=*C*H_2), 51.8 (*C*H_3), 37.9 (*C*H_2Ar). *HRMS* (ESI, positive, H_2O) m/z calcd. for $[C_{16}H_{19}O_4]^+$ 275.1278 found $[M+H]^+$ 275.1281, calcd. for $[C_{12}H_{22}NO_4]^+$ 292.1543 found $[M+NH_4]^+$ 292.1546, calcd. for $[C_{16}H_{18}NaO_4]^+$ 297.1097 found $[M+Na]^+$ 297.1099

Synthesis of 4: A solution of **2** (0.387 g, 1.41 mmol) in ethyl acetate (2 mL) was added dropwise to a solution of **3** (0.514 g, 2.82 mmol) in ethyl acetate (5 mL). The reaction was stirred at 25 °C for 4 months. The solvent was removed under reduced pressure at room temperature and the crude product was precipitated with THF. The solid was filtered off, washed with THF and dried under reduced pressure to afford compound **4** as a white solid (0.243 g, 30 %).

^{1}H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm) 7.19-7.12 (m, 1H, CH_{Ar}), 7.01-6.93 (m, 3H, CH_{Ar}), 4.50 (d, 2H, C*H*, *J* = 13.10 Hz), 4.32 (d, 2H, N-C*H*-N, *J* = 2.5 Hz), 3.54-3.41 (m, 4H, CH_2-Ar), 3.22 (d, 2H, N-C*H*-N, J = 2.5 Hz), 3.19-2.09 (m, 30H, C$H_2\alpha$N), 1.37-1.23 (m, 2H, C$H_2\beta$N). *^{13}C Jmod NMR* ($CDCl_3$, 75 MHz, 298 K) δ (ppm) 171.2 (*C*O), 139.7 (C_{ipso}), [129.6, 128.7, 127.0] (*C*H_{Ar}), [76.0, 70.6] (*C*H_{cis}), 55.7 (*C*H_2Ar), [54.1, 53.2, 52.9, 44.3, 44.2, 40.2] (*C*$H_2\alpha$N), 37.0 (*C*H), 35.3 (*C*$H_2\alpha$N), 19.5 (*C*$H_2\beta$N). *HRMS* (ESI, positive, H_2O)

m/z calcd. for $[C_{32}H_{47}N_8O_2]^+$ 575.3816 found $[M+H]^+$ 575.3819, calcd. for $[C_{32}H_{46}N_8NaO_2]^+$ 597.3636 found $[M+Na]^+$ 597.3640, calcd. for $[C_{32}H_{48}N_8O_2]^{2+}$ 288.1945 found $[M+2H]^{2+}$ 288.1949

Synthesis of 5: A suspension of NaH (0.5018 g, 20.91 mmol) in THF (10 mL) was cooled to 0 °C and diethyl malonate (3.257 g, 20.4 mmol) was added dropwise and stirring was continued until the solution became clear. Then *m*-dibromoxylene (1.500 g, 5.10 mmol) was added, and the solution was allowed to warm to room temperature and stirred for 1 h, then heated for 18 h at 50 °C. The reaction mixture was quenched with $NH_4Cl_{(sat)}$ and the product was extracted from the aqueous layer with ethyl acetate. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc, 100:0 to 0:100) to afford compound **5** as a colorless oil (1.1674 g, 54 %).

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K) δ (ppm) 7.20-7.15 (m, 1H, CH_{Ar}), 7.06-7.03 (m, 3H, CH_{Ar}), 4.20-4.10 (m, 8H, O-CH_2-CH_3), 3.59 (t, 2H, C*H*, J = 7.8 Hz), 3.16 (d, 4H, CH_2Ar, J = 7.8 Hz), 1.20 (t, 12H,O-CH_2-CH_3, J = 7.2 Hz). *^{13}C Jmod NMR* ($CDCl_3$, 125 MHz, 298 K) δ (ppm) 168.9 (*C*O), 138.3 (C_{ipso}), [129.4, 128.7, 127.3] (*C*H_{Ar}), 61.5 (O-*C*H_2-CH_3), 53.9 (*C*H), 34.6 (*C*H_2Ar), 14.1 (O-CH_2-*C*H_3). *HRMS* (ESI, positive, H_2O) m/z calcd. for $[C_{22}H_{31}O_8]^+$ 423.2013 found $[M+H]^+$ 423.2015, calcd. for $[C_{22}H_{30}NaO_8]^+$ 445.1832 found $[M+Na]^+$ 445.1837, calcd. for $[C_{22}H_{30}KO_8]^+$ 461.1572 found $[M+K]^+$ 461.1573

Synthesis of 6: A suspension of $LiAlH_4$ (0.7329 g, 19.4 mmol) in THF (25 mL) was cooled to -10 °C and a solution of **5** (1.3635 g, 3.23 mmol) in THF (7 mL) was added dropwise. The reaction mixture was warmed slowly to room temperature (~2 h) and heated to 50 °C for 18 h. Celite® and Et_2O were added before the addition of H_2O (1 mL), HCl (3M, 1 mL), H_2O (1 mL) and the mixture was filtered off. The filtrate was concentrated under reduced pressure to afford compound **6** as a colorless oil (0.7443 g, 91 %).

^{1}H NMR (MeOD, 400 MHz, 298 K) δ (ppm) 7.21-7.16 (m, 1H, CH_{Ar}), 7.09-7.01 (m, 3H, CH_{Ar}), 3.54 (d, 8H, CH_2-OH, J = 5.7 Hz), 2.62 (d, 4H, CH_2-Ar, J = 7.3 Hz), 1.94-1.86 (q, 2H, C*H*, *J* = 7.3, 5.7 Hz). *^{13}C Jmod NMR* (MeOD, 125 MHz, 298 K) δ (ppm) 141.8 (C_{ipso}), [131.1, 129.2, 127.8] (*C*H_{Ar}), 63.1 (*C*H_2-OH), 46.6 (*C*H), 35.1 (*C*H_2-Ar). *HRMS* (ESI, positive, H_2O) m/z calcd. for $[C_{14}H_{23}O_4]^+$ 255.1591 found $[M+H]^+$ 255.1594, calcd. for $[C_{14}H_{22}NaO_4]^+$ 277.1410 found 277.1415

Synthesis of 7: Compound **6** (0.350 g, 1.38 mmol) and triphenylphosphine (7.2075 g, 27.5 mmol) were solubilized in DMF (60 mL) and carbon tetrabromide (9.1275 g, 27.5 mmol) was added. The reaction was stirred at 30 °C for 18 h. The solvent was removed under reduced pressure. The crude product was dissolved in H_2O (40 mL) and extracted with dichloromethane (2 × 100 mL). Then, purification on silica gel was performed (Hexane/DCM 100:0 to 80:20) to afford compound **7** as a colorless oil (0.4362 g, 63 %).

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K) δ (ppm) 7.30 (m, 1H, CH_{Ar}), 7.14-7.08 (m, 3H, CH_{Ar}), 3.59 (dd, 4H, CH_2-Br, J = 10.5, 4.6 Hz), 3.43 (dd, 4H, CH_2-Br, J = 10.5, 6.3 Hz), 2.18 (d, 4H, CH_2-Ar, J = 7.5 Hz), 2.32-2.22 (m, 2H, C*H*). *^{13}C Jmod NMR* ($CDCl_3$, 125 MHz, 298 K) δ (ppm) 138.9 (C_{ipso}), [130.0, 129.3, 127.6] (CH_{Ar}), 43.8 (*C*H), 37.5 (CH_2-Ar), 35.8 (CH_2-Br). *MALDI-TOF* (matrix: dithranol): m/z calcd for $[C_{14}H_{19}Br_4]$ 506.820 found $[M+H]^+$ 506.254

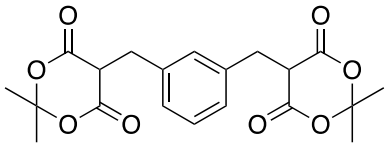
Synthesis of 9: To a solution of **7** (0.4362 g, 0.86 mmol) in dry acetonitrile (7.5 mL), a solution of compound **8** (0.372 g, 1.77 mmol) in dry acetonitrile (7.5 mL) was added dropwise. Then, K_2CO_3 (2.3770 g, 17.2 mmol, 20 equiv.) was added and the reaction was stirred at 60 °C for 10 days. K_2CO_3 was removed through filtration with cannula and filtrate was concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel (DCM/Et_3N/MeOH 97:3:0 to 87:3:10) to afford compound **9** as a white foam (0.0836 g, 16 %).

^{1}H NMR ($CDCl_3$, 500 MHz, 298 K) δ (ppm) 7.15-7.07 (m, 1H, CH_{Ar}), 6.99-6.81 (m, 3H, CH_{Ar}), 3.98 (br, 1H), 3.82-3.58 (m,3H), 3.44-3.02 (m, 5H), 2.90-2.11 (m, 31H, C$H_2\alpha$N), 1.75 (br, 1H), 1.27 (s, 6H, CH_3), 1.22 (s, 6H, CH_3), 1.17-1.07 (m, 2H, C$H_2\beta$N). *^{13}C Jmod NMR* ($CDCl_3$, 125 MHz, 298 K) δ (ppm) [142.0 (x2), 141.9, 139.6 (x2)] (C_{ipso}), [129.8, 129.7, 129.5, 128.3, 128.2, 126.7, 126.6, 126.4, 126.3] (CH_{Ar}), 74.1 (N-*C*(CH_3)-N), [55.6, 53.1, 53.0, 50.9, 50.9, 50.8, 50.7, 49.3, 46.7, 46.0, 45.0, 45.0, 44.9] ($CH_2\alpha$N), 39.1 ($CH_2\gamma$N), [34.4, 28.7] (*C*H), [18.1, 18.0] ($CH_2\beta$N), [11.3, 11.1, 10.9, 10.0] (CH_3). *HRMS* (ESI, positive, H_2O) m/z calcd. for $[C_{36}H_{59}N_8]^+$ 603.4857 found $[M+H]^+$ 603.4853, calcd. for $[C_{36}H_{60}N_8]^{2+}$ 302.2465 found $[M+2H]^{2+}$ 302.2466, calcd. for $[C_{36}H_{61}N_8]^{3+}$ 201.8334, found $[M+3H]^{3+}$ 201.8336.

Synthesis of 10: Compound **9** (0.0836 g, 0.139 mmol) was solubilized in HCl (3 M, 14 mL) and the reaction was stirred at room temperature for 24 h. An extraction with $CHCl_3$ (3 × 20 mL) was performed to remove organic impurities. Then the aqueous layer was concentrated under reduced pressure and the product was purified by flash chromatography on reversed-phase C18 silica (H_2O(0.1 % formic acid)/CH_3CN 100:0 to 0:100) to afford compound ***C,C'*-(*m*-xylylene)bis-cyclam** as a brown film (0.071 g, 59 %).

^{1}H NMR (D_2O, 500 MHz, 298 K) δ (ppm) 8.18 (s, 0.3H, *H*COOH), 7.38 (m, 1H, CH_{Ar}), 7.31-7.22 (m, 3H, CH_{Ar}), 3.70-3.54 (m, 13H), 3.53-3.33 (m, 20H), 3.28-3.18 (m, 6H), 3.00-2.85 (m, 6H), 2.28 (br, 4H, C$H_2\beta$N). *^{13}C Jmod NMR* (D_2O, 125 MHz, 298 K) δ (ppm) 140.3 (C_{ipso}), [132.9, 132.4, 131.0] (CH_{Ar}), [50.5, 45.4, 44.5, 43.5] ($CH_2\alpha$N), 38.4 ($CH_2\gamma$N), 36.1 (*C*H), 22.6 ($CH_2\beta$N). *HRMS* (ESI, positive, H_2O) m/z calcd. for $[C_{28}H_{56}N_8]^+$ 252.2308 found $[M+2H]^{2+}$ 252.2309, calcd. for $[C_{28}H_{57}N_8]^{3+}$ 168.4897 found $[M+3H]^{3+}$ 168.4902, calcd. for $[C_{28}H_{58}N_8]^{4+}$ 126.6191 found $[M+4H]^{4+}$ 126.6192.

Spectral data
Compound 1:



Formula: $C_{20}H_{22}O_8$

Molecular Weight: 390.39 $g.mol^{-1}$

Description: white powder

Yield: 85%

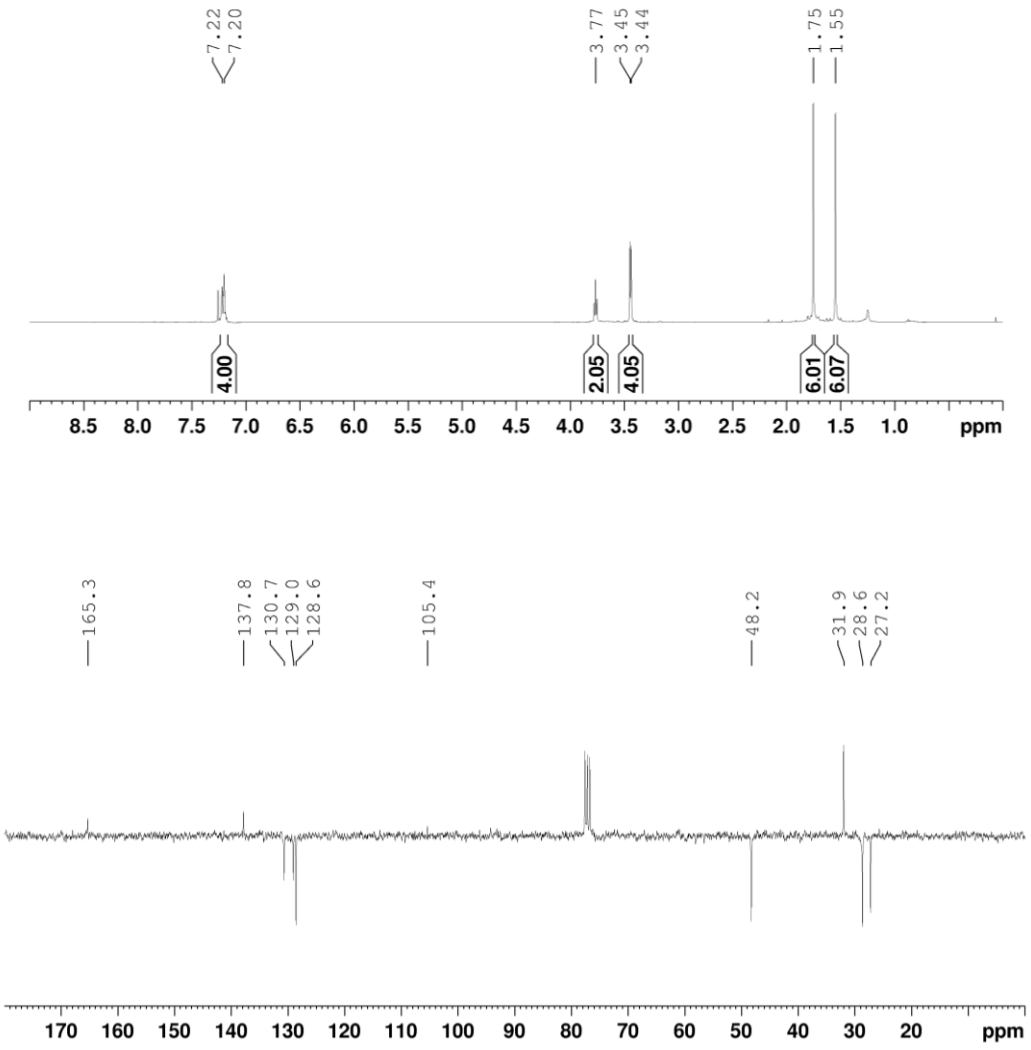


Figure S1: 1H (400 MHz, CDCl3, 298K, TMS) and 13C Jmod (75 MHz, CDCl3, 298K, TMS) NMR

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Plate-forme de Spectrométrie de Masse Haute Résolution

HRAM

Analysis Info

Sample Name	**MLR087**	Acquisition Date	30/09/2020 14:41:04
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		Method	Positif.m

Acquisition Parameter

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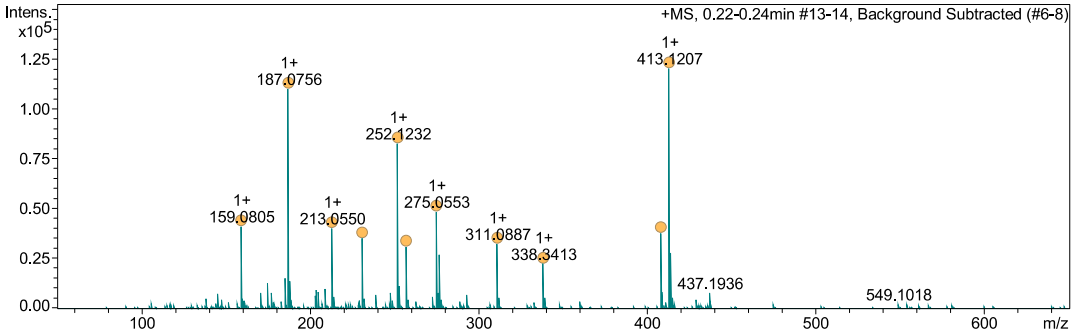

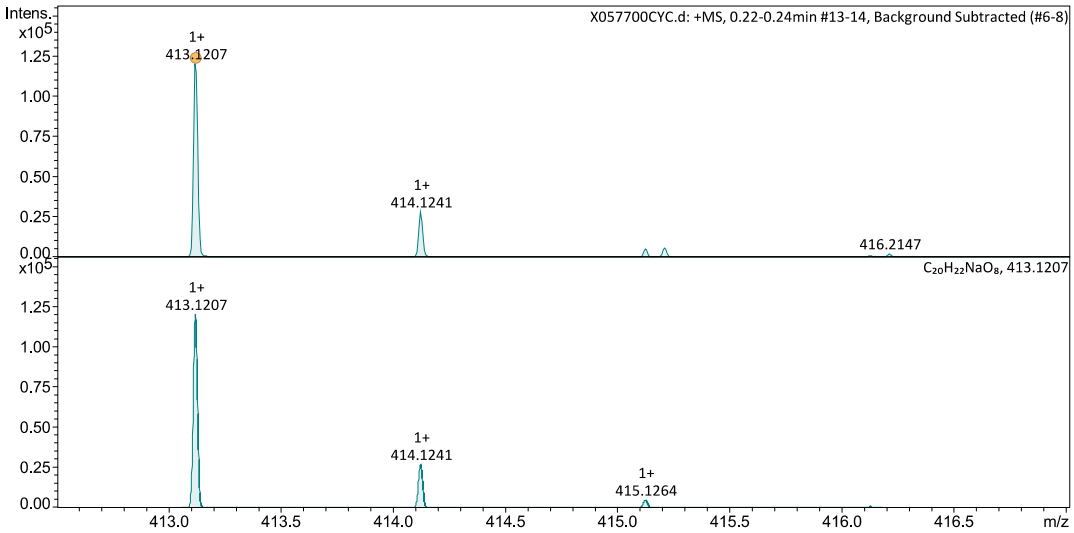

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e¯ Conf
159.080450	1+	1	C11H11O	159.080441	-0.1	5.0	7.0	even
187.075625	1+	1	C12H11O2	187.075356	-1.4	3.0	8.0	even
213.055019	1+	1	C13H9O3	213.054621	-1.9	6.9	10.0	even
231.065468	1+	1	C13H11O4	231.065185	-1.2	4.5	9.0	even
252.123189	1+	1	C13H18NO4	252.123034	-0.6	4.4	6.0	even
257.044711	1+	1	C14H9O5	257.044450	-1.0	12.9	11.0	even
275.055283	1+	1	C14H11O6	275.055014	-1.0	8.2	10.0	even
311.088689	1+	1	C16H16NaO5	311.088994	1.0	14.6	9.0	even

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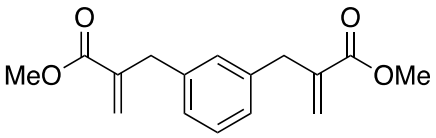
***Figure S2**: HRMS spectrum (ESI)*

Compound 2:



Formula: $C_{16}H_{18}O_4$

Molecular Weight:274.32 g.mol^{-1}

Description: orange oil

Yield: 97%

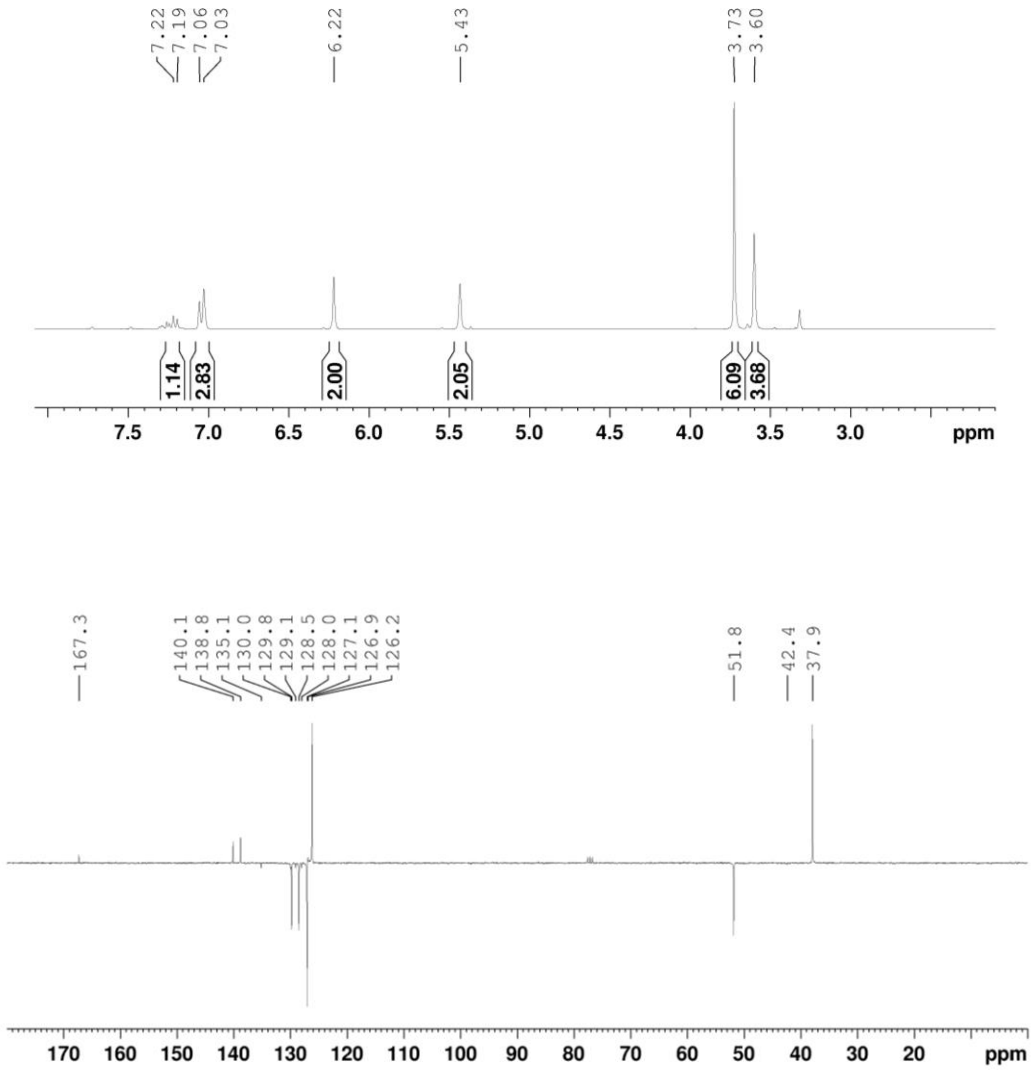
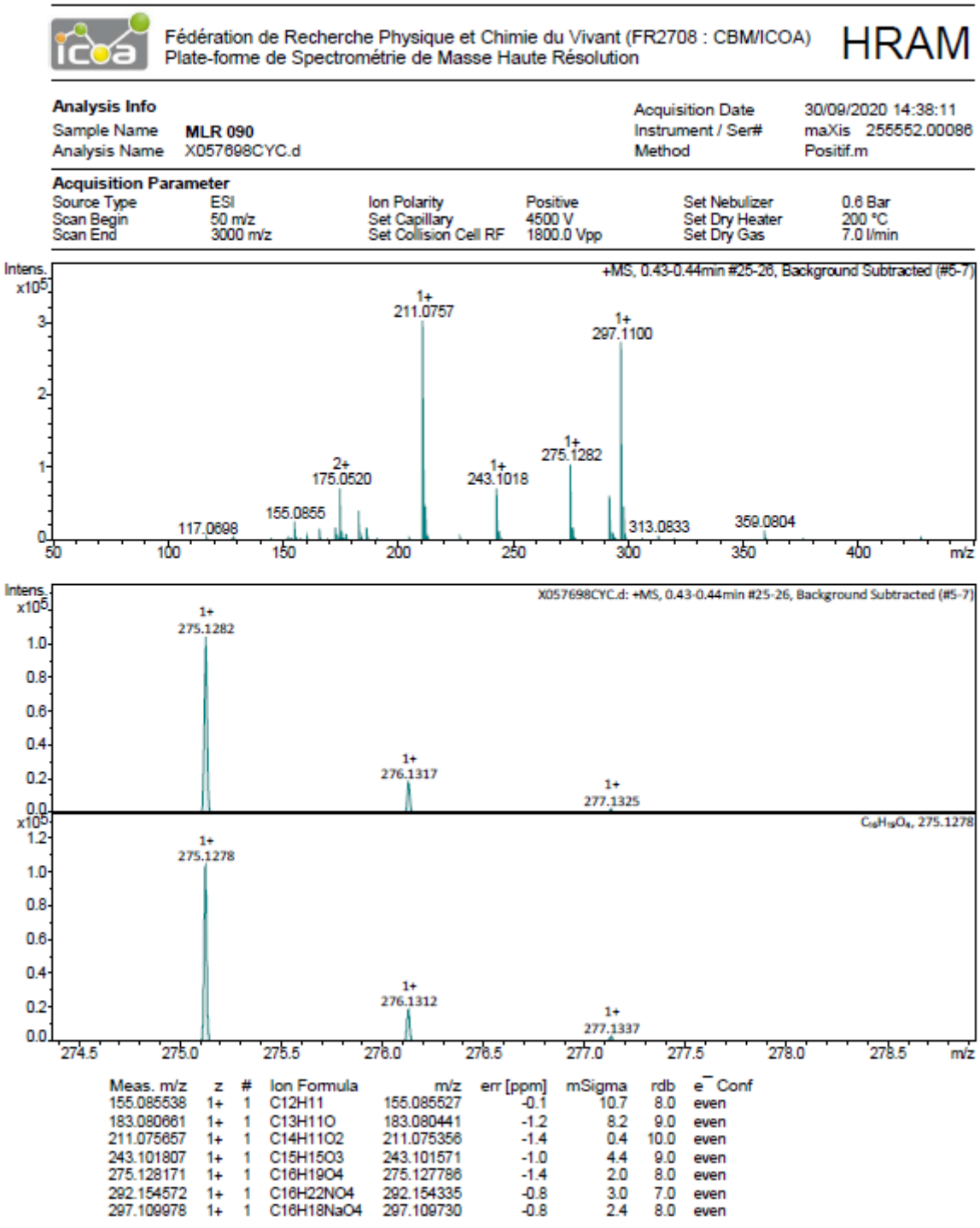


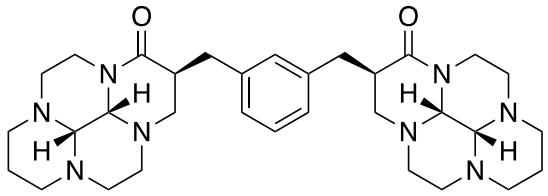
Figure S3 : *^{1}H (300 MHz, $CDCl_3$, 298K, TMS) and ^{13}C Jmod (75 MHz, $CDCl_3$, 298K, TMS) NMR*



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
155.085538	1+	1	C12H11	155.085527	-0.1	10.7	8.0	even
183.080661	1+	1	C13H11O	183.080441	-1.2	8.2	9.0	even
211.075657	1+	1	C14H11O2	211.075356	-1.4	0.4	10.0	even
243.101807	1+	1	C15H15O3	243.101571	-1.0	4.4	9.0	even
275.128171	1+	1	C16H19O4	275.127786	-1.4	2.0	8.0	even
292.154572	1+	1	C16H22NO4	292.154335	-0.8	3.0	7.0	even
297.109978	1+	1	C16H18NaO4	297.109730	-0.8	2.4	8.0	even

***Figure S4**: HRMS spectrum (ESI)*

Compound 4:



Formula: $C_{32}H_{46}N_8O_2$

Molecular Weight: 574.77 g.mol^{-1}

Description: white solid

Yield: 30 %

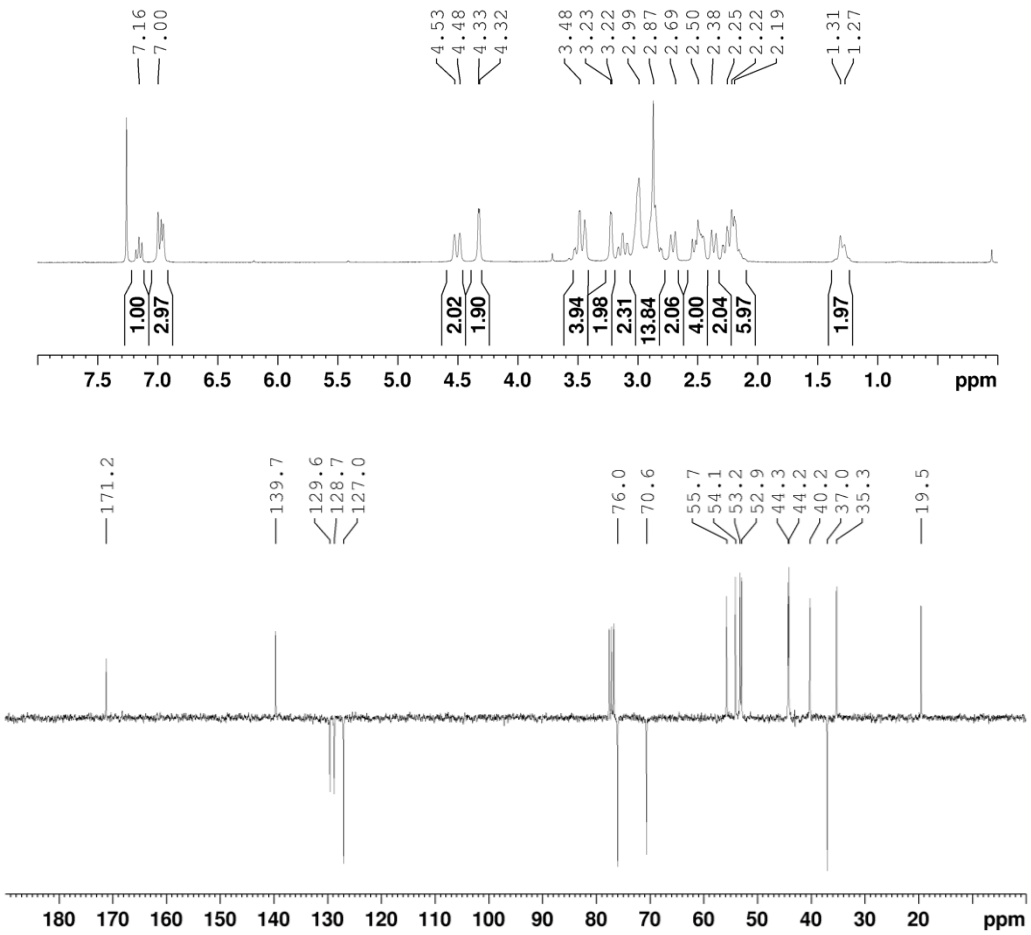


Figure S5: *^{1}H (300 MHz, $CDCl_3$, 298K, TMS) and ^{13}C Jmod (75 MHz, $CDCl_3$, 298K, TMS) NMR*

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Plate-forme de Spectrométrie de Masse Haute Résolution

HRAM

Analysis Info

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		Method	Positif.m

Acquisition Parameter

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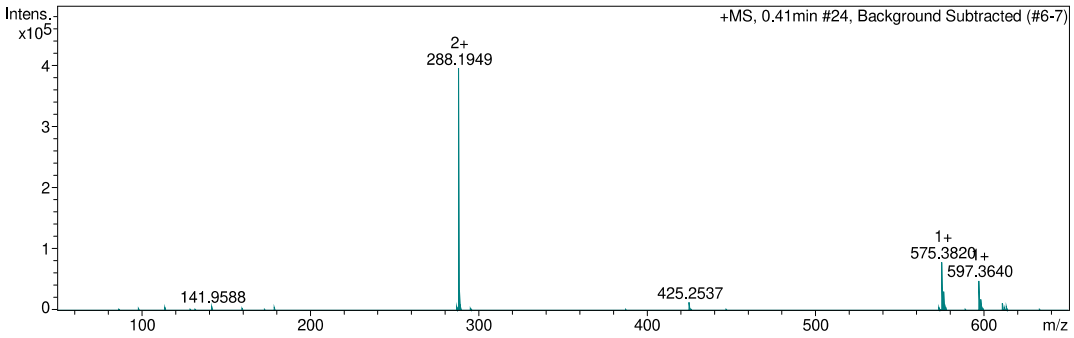
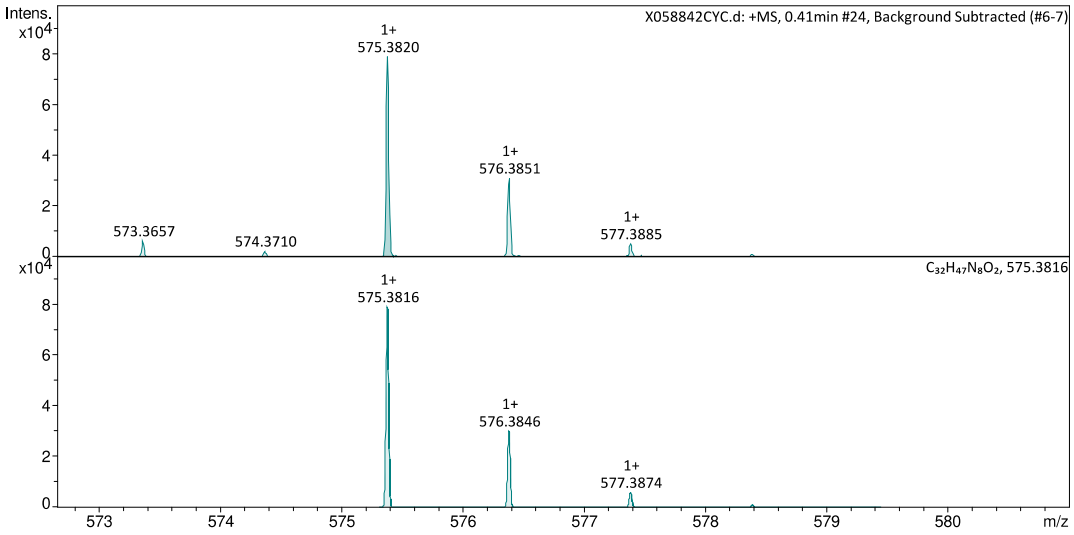
Scan End	3000 m/z	Set Collision Cell RF	1800.0 Vpp	Set Dry Gas	7.0 l/min





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
288.194939	2+	1	C32H48N8O2	288.194463	-1.7	14.3	14.0	even
575.381999	1+	1	C32H47N8O2	575.381649	-0.6	9.8	14.0	even
597.364017	1+	1	C32H46N8NaO2	597.363593	-0.7	11.1	14.0	even

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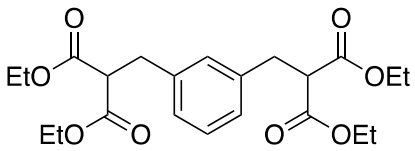
***Figure S6**: HRMS spectrum (ESI)*

Table S1: *X-ray data of compound 4*

Empirical formula	$C_{32}H_{46}N_8O_2, C_4H_8O_2$
Formula weight	662.87
Temperature	193(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	$P\ \bar{1}$
Unit cell dimensions	a = 10.1829(8) Å α = 63.913(2)° b = 13.8753(10) Å β = 89.426(2)° c = 14.2627(10) Å γ = 73.285(2)°
Volume	1717.5(2) $Å^3$
Z	2
Density (calculated)	1.282 Mg/m^3
Absorption coefficient	0.086 mm^{-1}
F(000)	716
Crystal size	0.200 x 0.180 x 0.100 mm^3
Theta range for data collection	1.604 to 29.155°
Index ranges	-13<=h<=13, -17<=k<=18, -14<=l<=19
Reflections collected	53091
Independent reflections	9117 [R(int) = 0.0568]
Completeness to theta = 25.242°	99.5 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7458 and 0.6737
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	9117 / 0 / 435
Goodness-of-fit on F^2	1.032
Final R indices [I>2sigma(I)]	R1 = 0.0797, wR2 = 0.2293
R indices (all data)	R1 = 0.1323, wR2 = 0.2686
Extinction coefficient	n/a
Largest diff. peak and hole	0.780 and -0.578 $e.Å^{-3}$

Compound 5:



Formula: $C_{22}H_{30}O_9$

Molecular Weight: 422.47 g.mol^{-1}

Description: colorless oil

Yield: 54 %

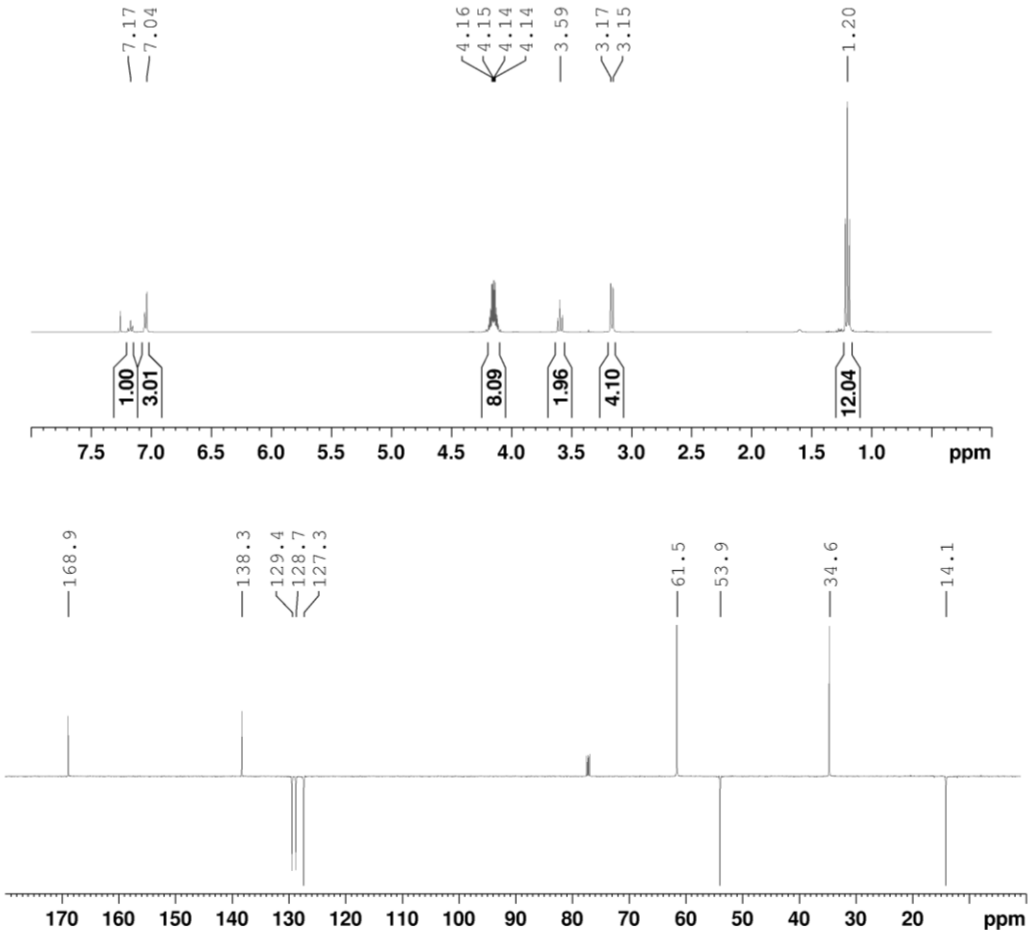


Figure S7: *^{1}H (400 MHz, $CDCl_3$, 298K, TMS) and ^{13}C Jmod (125 MHz, $CDCl_3$, 298K, TMS) NMR*

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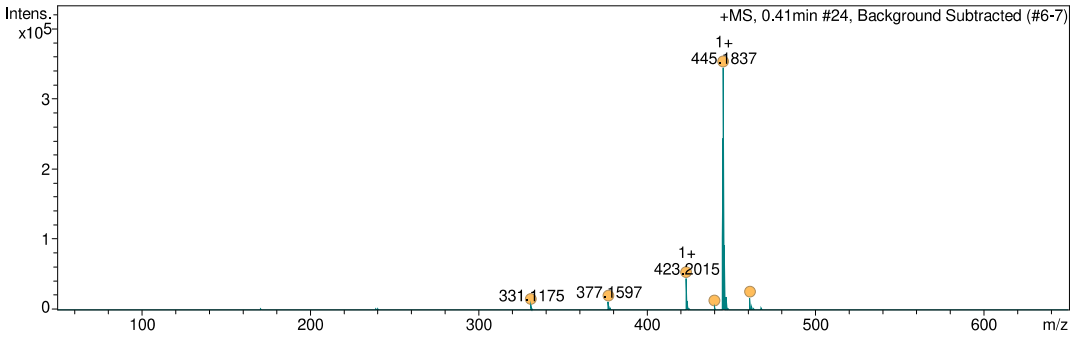
HRAM

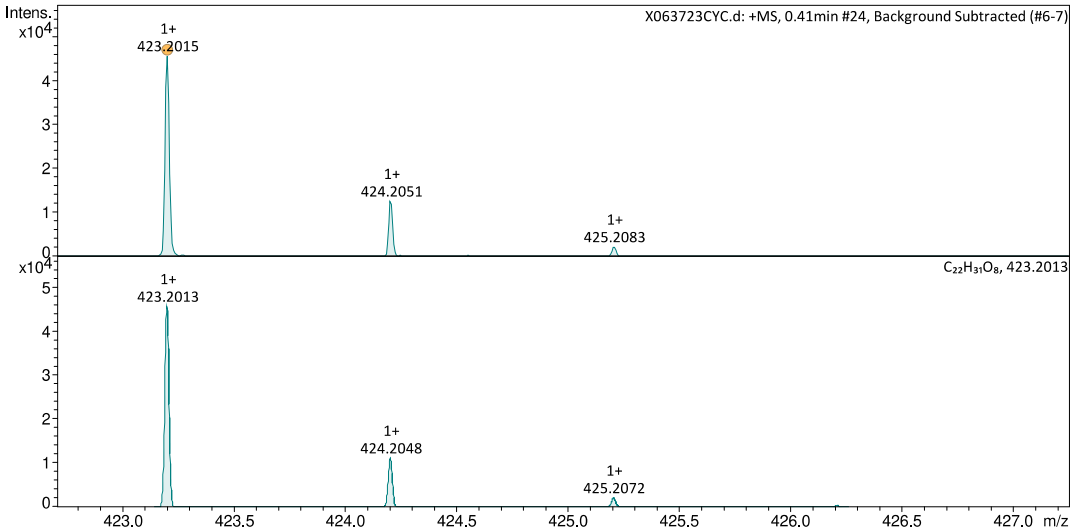
Analysis Info

Sample Name	**MLR 194**	Acquisition Date	01/12/2021 18:25:31
Analysis Name	X063723CYC.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	3000 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
331.117474	1+	1	C18H19O6	331.117615	0.4	32.5	10.0	even
377.159659	1+	1	C20H25O7	377.159480	-0.5	27.7	9.0	even
423.201542	1+	1	C22H31O8	423.201344	-0.5	16.5	8.0	even
440.228285	1+	1	C22H34NO8	440.227893	-0.9	n.a.	7.0	even
445.183744	1+	1	C22H30NaO8	445.183289	-1.0	11.9	8.0	even
461.157381	1+	1	C22H30KO8	461.157226	-0.3	26.6	8.0	even

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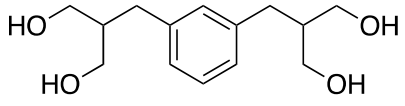
***Figure S8**: HRMS spectrum (ESI)*

Compound 6:



Formula: $C_{14}H_{22}O_4$

Molecular Weight: 254.33 $g.mol^{-1}$

Description: colorless oil

Yield: 91%

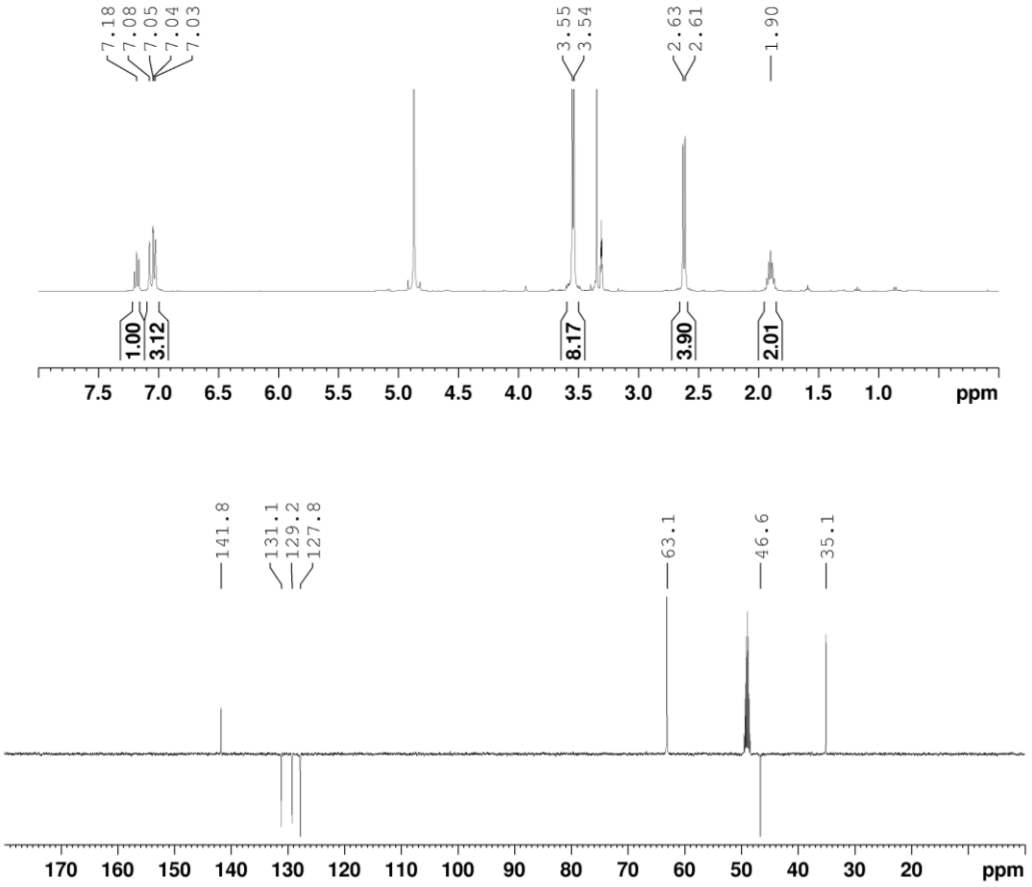


Figure S9: *[1]H (400 MHz, MeOD, 298K, TMS) and [13]C Jmod (125 MHz, MeOD, 298K, TMS) NMR*

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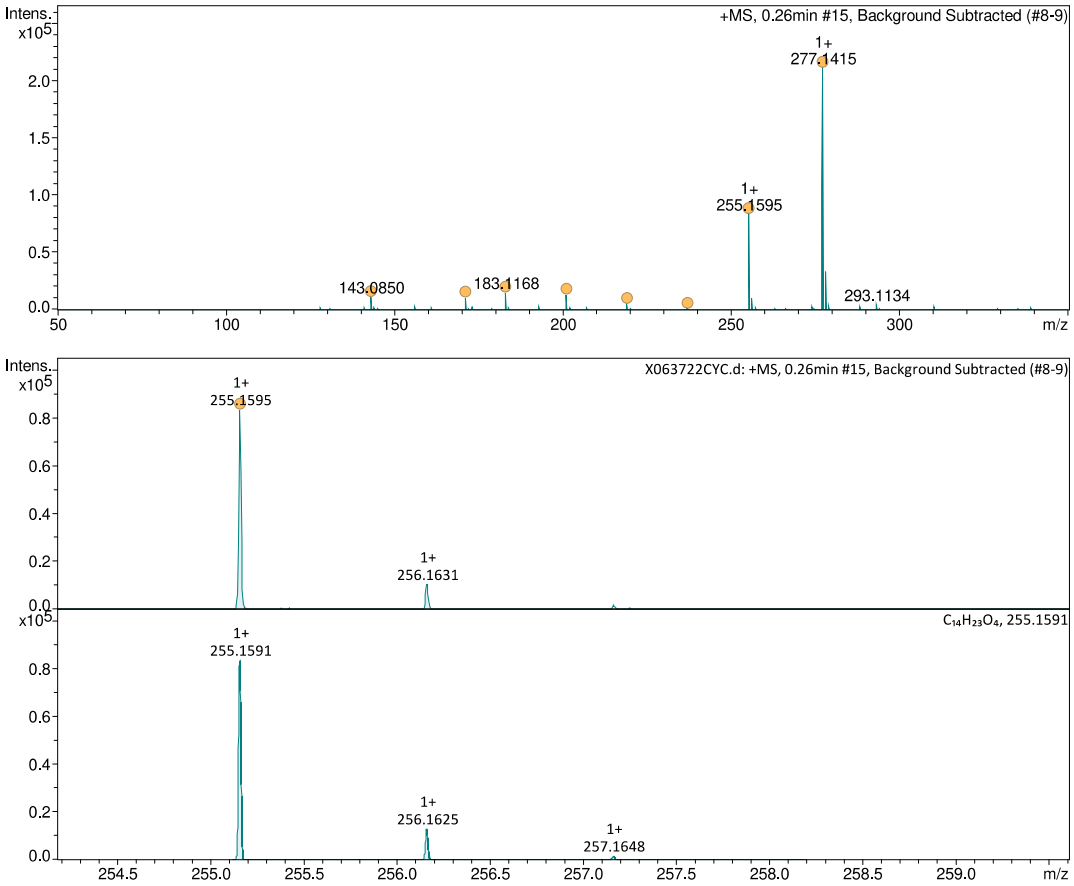
HRAM

Analysis Info

Sample Name	**MLR 195 f4**	Acquisition Date	01/12/2021 18:24:05
Analysis Name	X063722CYC.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	3000 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
143.085010	1+	1	C11H11	143.085527	3.6	35.1	7.0	even
171.116683	1+	1	C13H15	171.116827	0.8	124.5	7.0	even
183.116782	1+	1	C14H15	183.116827	0.2	6.4	8.0	even
201.127602	1+	1	C14H17O	201.127392	-1.0	8.5	7.0	even
219.138264	1+	1	C14H19O2	219.137956	-1.4	n.a.	6.0	even
237.147600	1+	1	C14H21O3	237.148521	3.9	n.a.	5.0	even
255.159479	1+	1	C14H23O4	255.159086	-1.5	21.6	4.0	even
277.141462	1+	1	C14H22NaO4	277.141030	-1.6	3.0	4.0	even

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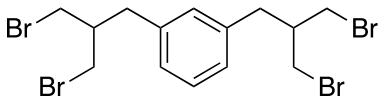
Figure S10**: HRMS spectrum (ESI) of compound **6

Compound 7:



Formula: $C_{14}H_{18}Br_4$

Molecular Weight: 505.91 $g.mol^{-1}$

Description: colorless oil

Yield: 63 %

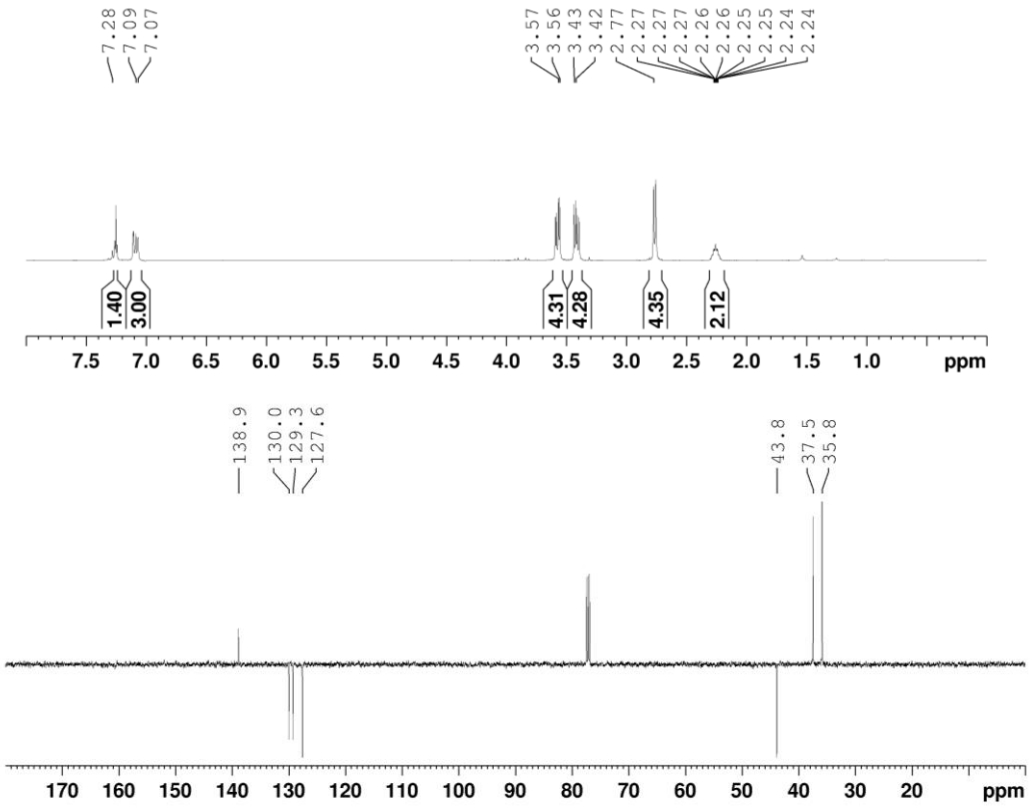
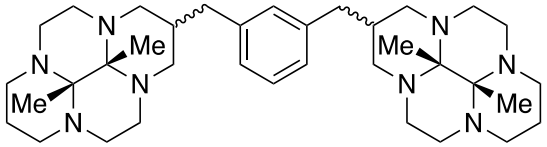


Figure S11: *^{1}H (400 MHz, $CDCl_3$, 298K, TMS) and ^{13}C Jmod (125 MHz, $CDCl_3$, 298K, TMS) NMR*

Compound 9 (mixture of diastereomers):



Formula: $C_{36}H_{58}N_8$

Molecular Weight: 602.92 g.mol^{-1}

Description: white foam

Yield: 16 %

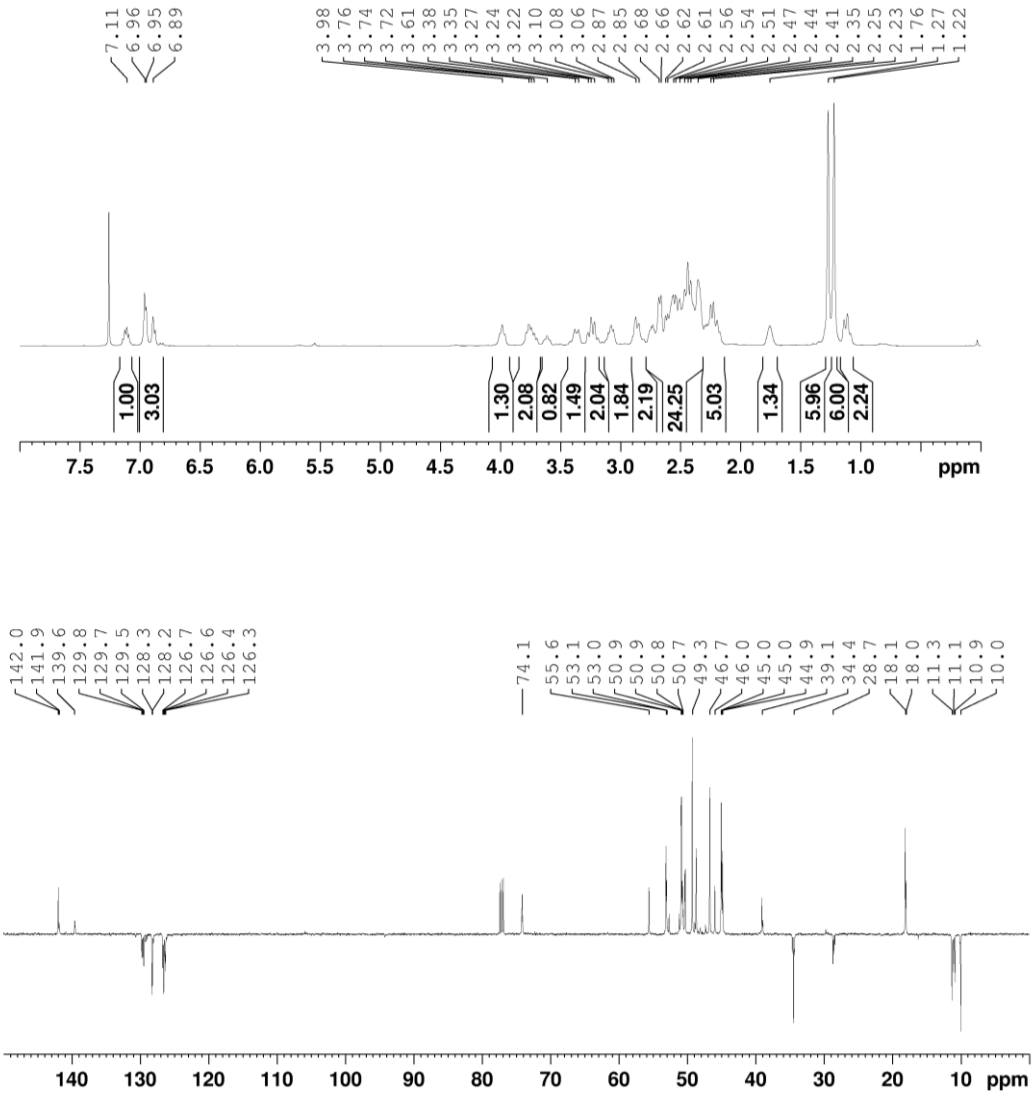


Figure S12: *^{1}H (500 MHz, $CDCl_3$, 298K, TMS) and ^{13}C Jmod (125 MHz, $CDCl_3$, 298K, TMS) NMR*

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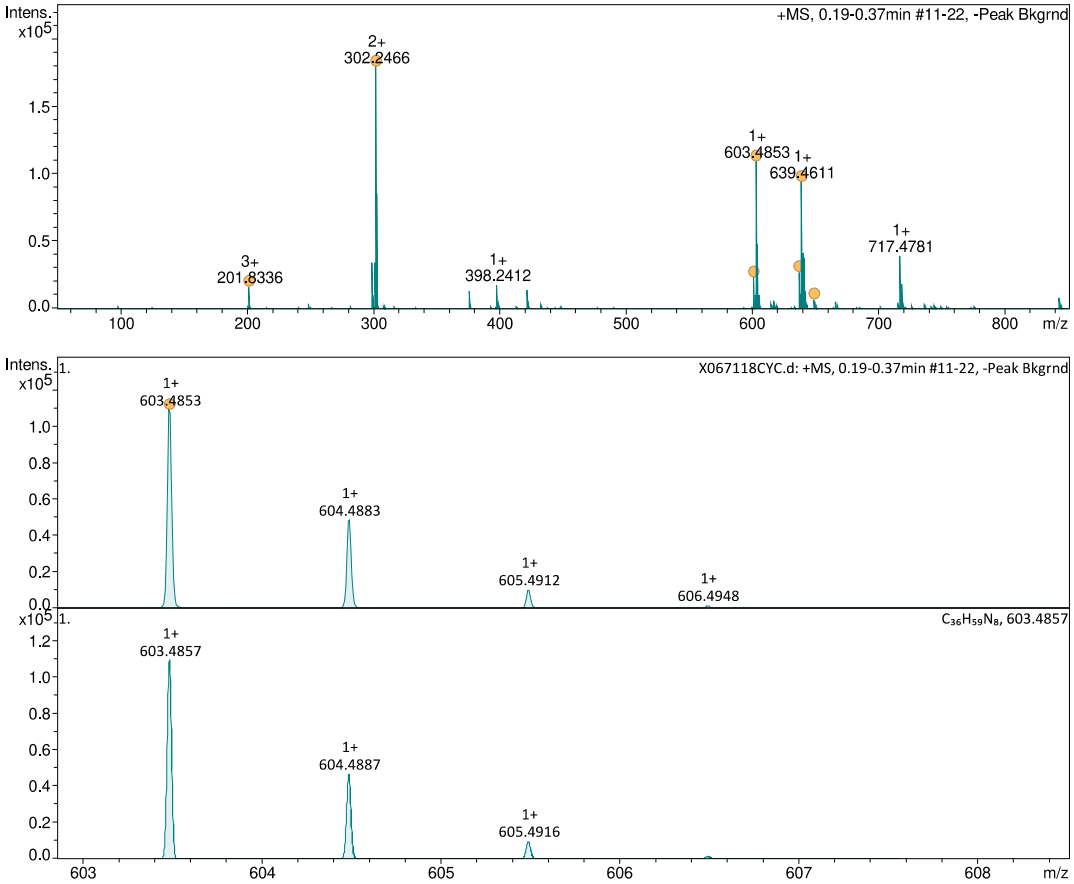
HRAM

Analysis Info

Sample Name	**MLR 255**	Acquisition Date	01/06/2022 12:37:39
Analysis Name	X067118CYC.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	3000 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
201.833568	3+	1	C36H61N8	201.833424	-0.7	14.3	12.0	even
302.246641	2+	1	C36H60N8	302.246498	-0.5	26.9	12.0	even
601.469153	1+	1	C36H57N8	601.470070	1.5	48.5	13.0	even
	1+	2	C35H61N4O4	601.468733	-0.7	621.4	8.0	even
603.485257	1+	1	C36H59N8	603.485720	0.8	9.1	12.0	even
	1+	2	C35H63N4O4	603.484383	-1.4	20.6	7.0	even
637.446645	1+	1	C35H62ClN4O4	637.445411	-1.9	193.3	7.0	even
	1+	2	C36H58ClN8	637.446748	0.2	194.7	12.0	even

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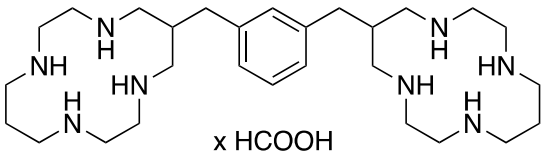
***Figure S13**: HRMS spectrum (ESI)*

Compound 10 (*C,C'*-(*m*-xylylene)bis-cyclam)



Formula: $C_{28}H_{54}N_8$

Molecular Weight: 871.0 g.mol^{-1} (for x = 8)

Description: brown film

Yield: 59 %

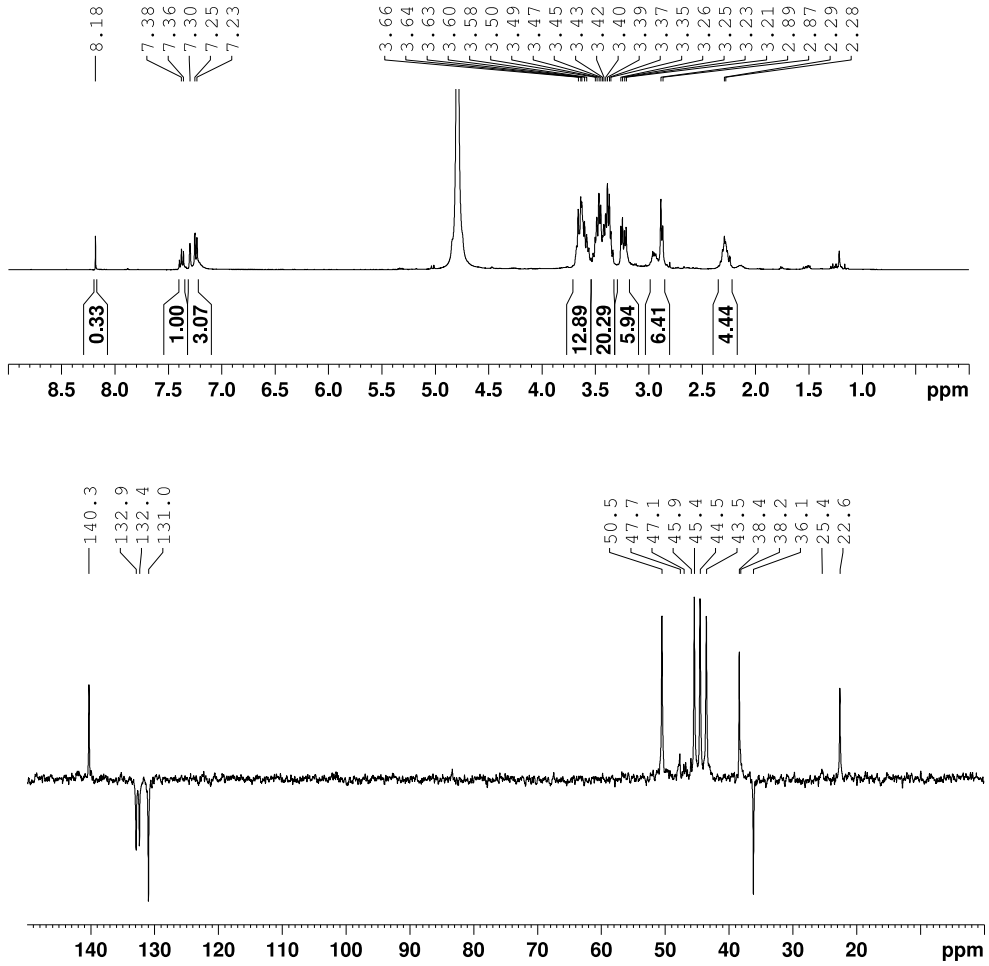


Figure S14: *^{1}H (400 MHz, D_2O, 298K, TMS) and ^{13}C Jmod (125 MHz, D_2O, 298K, TMS) NMR*

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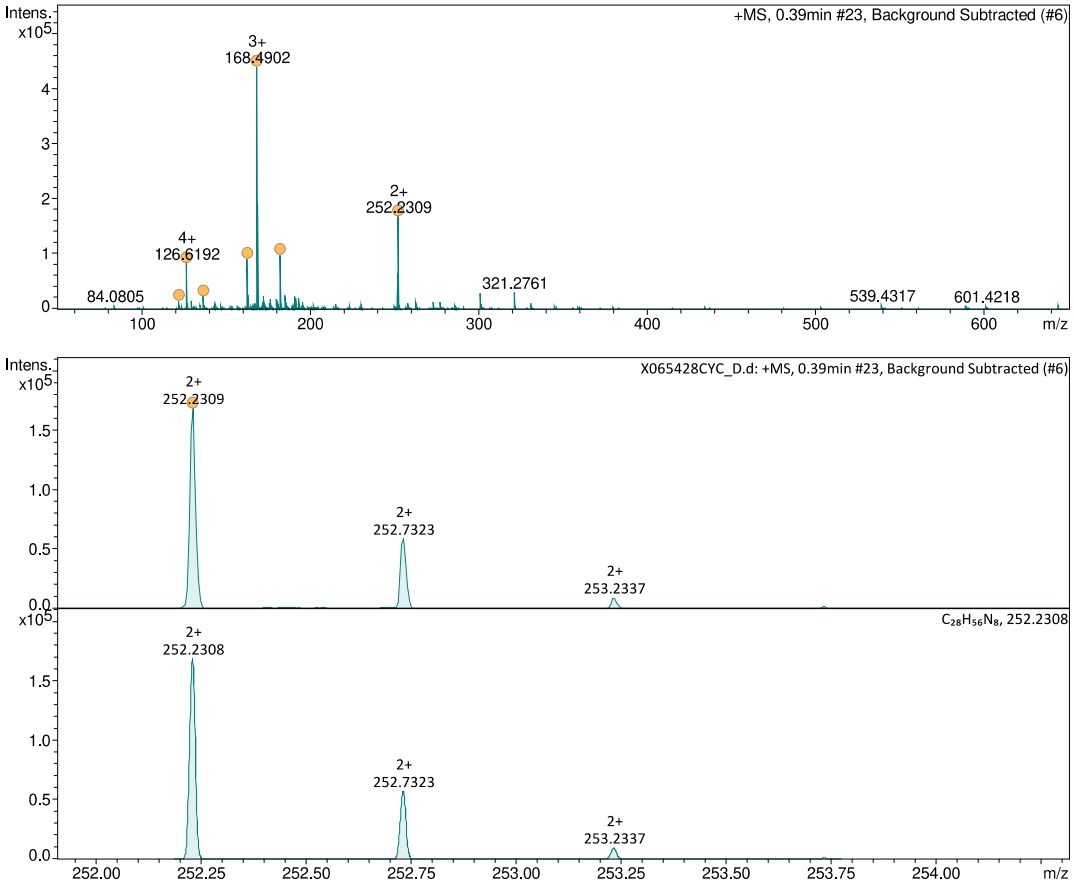
HRAM

Analysis Info

Sample Name	**MLR 215 f1**	Acquisition Date	23/02/2022 14:41:40
Analysis Name	X065428CYC_D.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	3000 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
122.362738	4+	1	C28H55N7	122.362425	-2.6	37.5	7.0	even
126.619239	4+	1	C28H58N8	126.619062	-1.4	45.5	6.0	even
136.875549	4+	1	C30H61N9	136.875700	1.1	12.0	7.0	even
162.814381	3+	1	C28H54N7	162.814141	-1.5	23.4	7.0	even
168.490208	3+	1	C28H57N8	168.489658	-3.3	56.1	6.0	even
182.165273	3+	1	C30H60N9	182.165174	-0.5	52.6	7.0	even
252.230881	2+	1	C28H56N8	252.230848	-0.1	5.5	6.0	even

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Figure S15: *HRMS spectrum (ESI)*

Nb: Compound 10 is not UV-active at the concentrations used for HPLC identification, therefore detection was performed with ELSD.

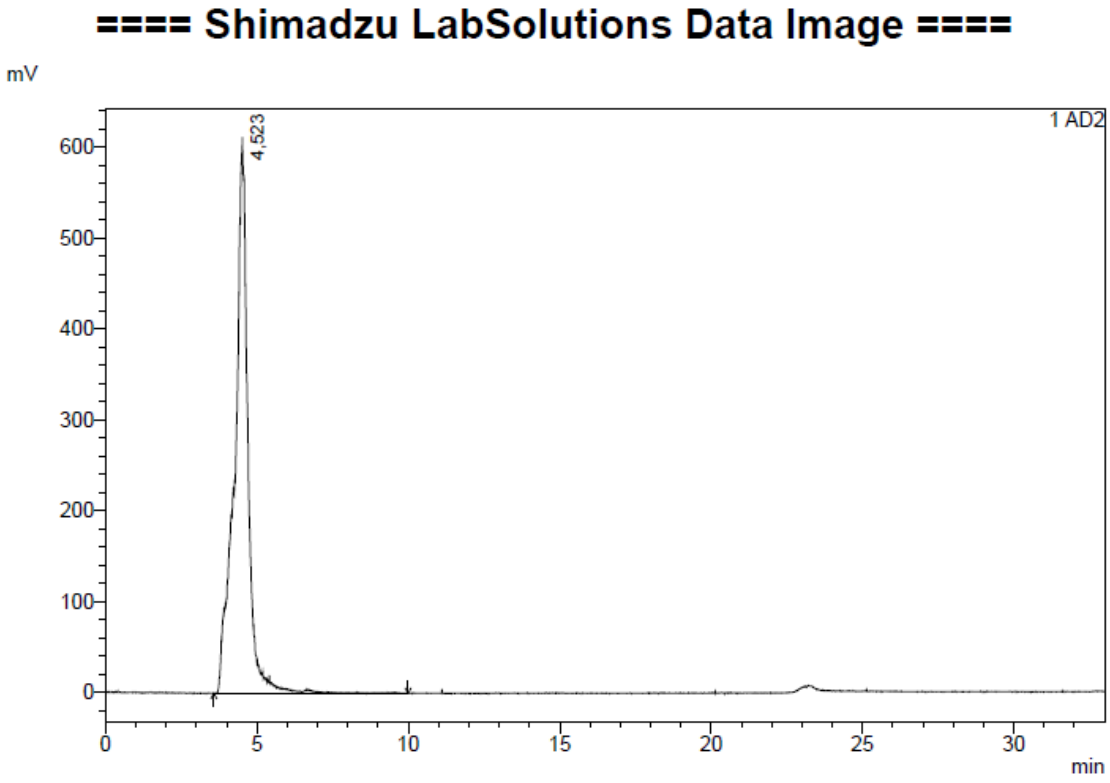


Figure S16: *HPLC-ELSD chromatogram. Mobile phase H_20 + 0.1% formic acid / Acetonitrile (95:5 for 16 mins, gradient to 10:90 in 6 mins + 2 mins at 10:90, gradient to 95:5 in 6 mins)*

Biological Assays

Table S2: *Calcium Signalling Assays (3 replicates, U87.CD4.CXCR4 cells)*

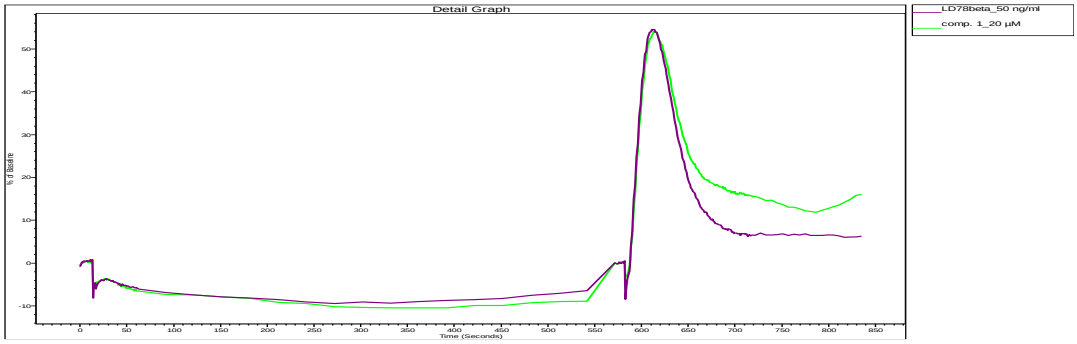
n = 1					
Group Name	**Concentration (nM)**	**Average**	**Minus Neg Contrl**	**%inhibition**	**IC50**
Negative Control		15,58	0		
CXCL12		91,99	76,41		
compound 10	50 µM	14,36	-1,22	101,60	
	10 µM	30,81	15,23	80,07	
	2 µM	56,32	40,74	46,68	2,34 µM
	0,4 µM	88,17	72,59	5,00	
	0,08 µM	90,95	75,37	1,36	
	0,016 µM	80,96	65,38	14,44	
	0,0032 µM	85,77	70,19	8,14	
	0,00064 µM	74,91	59,33	22,35	
AMD3100	2000 nM	16,7	1,12	98,53	
	400 nM	40,7	25,12	67,12	146,38 nM
	80 nM	61,65	46,07	39,71	
	16 nM	83,25	67,67	11,44	

n = 2					
Group Name	**Concentration (nM)**	**Average**	**Minus Neg Contrl**	**%inhibition**	**IC50**
Negative Control		19,24	0,00		
CXCL12		101,07	81,83		
compound 10	50 µM	14,91	-4,33	105,29	
	10 µM	29,93	10,69	86,94	
	2 µM	89,6	70,36	14,02	4,43 µM
	0,4 µM	105,57	86,33	-5,5	
	0,08 µM	102,25	83,01	-1,44	
	0,016 µM	105,73	86,49	-5,69	
	0,0032 µM	116,5	97,26	-18,86	
	0,00064 µM	122,12	102,88	-25,77	
AMD3100	2000 nM	22,69	3,45	95,78	
	400 nM	35,08	15,84	80,65	133,75 nM
	80 nM	71,92	52,68	35,62	
	16 nM	90,92	71,68	12,41	

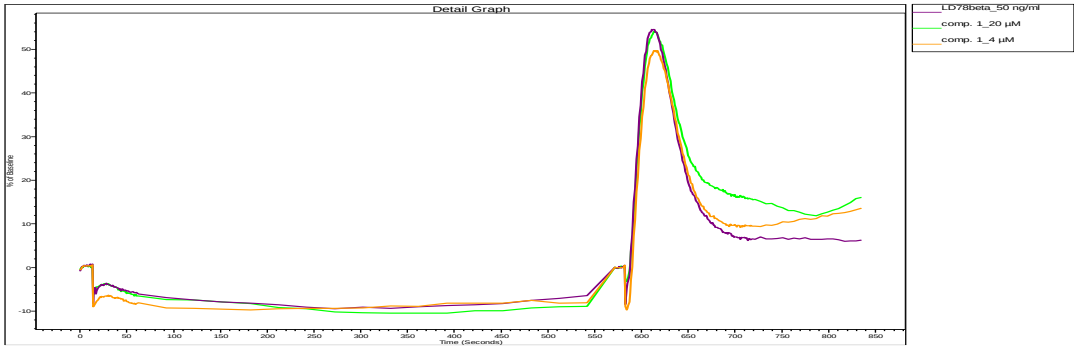
n = 3					
Group Name	**Concentration (nM)**	**Average**	**Minus Neg Contrl**	**%inhibition**	**IC50**
Negative Control		19,47	0		
CXCL12		93,25	73,78		
compound 10	50 µM	18,8	-0,67	100,91	
	10 µM	34,27	14,8	79,94	
	2 µM	72,81	53,34	27,70	3,98 µM
	0,4 µM	93,5	74,03	-0,34	
	0,08 µM	87,93	68,46	7,21	
	0,016 µM	92,8	73,33	0,61	

Figure S17: *Calcium Signalling Assays (U87.CD4.CCR5 cells)*

Compound 10 (20 µM)+ LD78beta (50 ng/ml)



Compound 10 (20 µM- 4 µM)+ LD78beta (50 ng/ml)



Maraviroc (8 nM- 1.6 nM-0.32 nM) + LD78beta (50 ng/ml) – **Positive control**

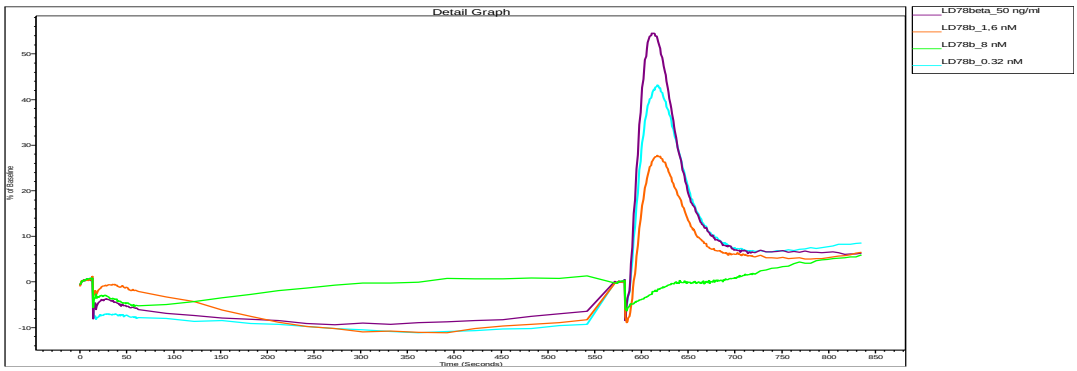


Figure S18: *CXCL12 inhibition Assay (Jurkat cells)*

	NC Average	96,5	78,6		
	PC average	929,0			
220715					
Name	conc.	Mean	SD	% Inh	IC-50 nM
AMD3100	PC	965	602	-4	
AMD3100	600	142	93,7	95	
AMD3100	120	236	135	83	34,2
AMD3100	24	591	357	41	
AMD3100	4,8	933	588	0	
AMD3100	0,96	1023	641	-11	
AMD3100	0,192	1001	631	-9	
AMD3100	NC	97,4	79,2	100	
cmpd 10	PC	893	558	4	
cmpd 10	10000	123	85,6	97	
cmpd 10	2000	138	91,7	95	
cmpd 10	400	300	172	76	193,9
cmpd 10	80	773	472	19	
cmpd 10	16	970	611	-5	
cmpd 10	3,2	966	606	-4	
cmpd 10	0,64	967	611	-5	

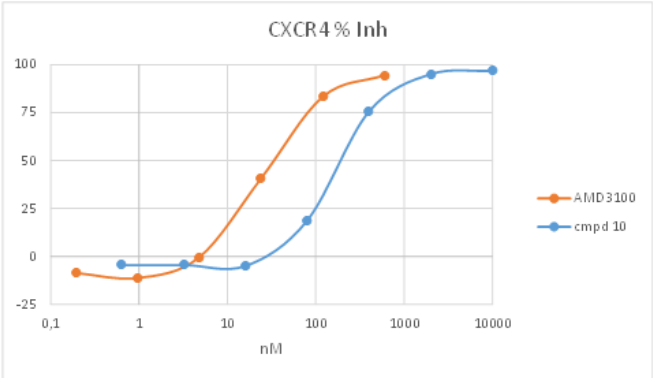


Table S3: *Monoclonal Antibodies (mAbs) binding assays (IC_{50} values, Jurkat cells)*

12G5

			AVERAGE
	IC_{50} (nM)	IC_{50} (nM)	**IC_{50} (nM)**
compound 10	341,3	411,7	**376,5**
AMD3100	6,8	6,8	**6,8**

44717

			AVERAGE
	IC_{50} (nM)	IC_{50} (nM)	**IC_{50} (nM)**
compound 10	954,1	405,6	**679,9**
AMD3100	110,0	16,6	**63,3**

1D9

			AVERAGE
	IC_{50} (nM)	IC_{50} (nM)	**IC_{50} (nM)**
compound 10	> 12 000	> 20 000	**> 20 000**
AMD3100	> 12 000	> 20 000	**> 20 000**

2B11

			AVERAGE
	IC_{50} (nM)	IC_{50} (nM)	**IC_{50} (nM)**
compound 10	> 12 000	> 20 000	**> 20 000**
AMD3100	> 12 000	> 20 000	**> 20 000**

Figure S19: *Monoclonal Antibodies (mAbs) binding assays*

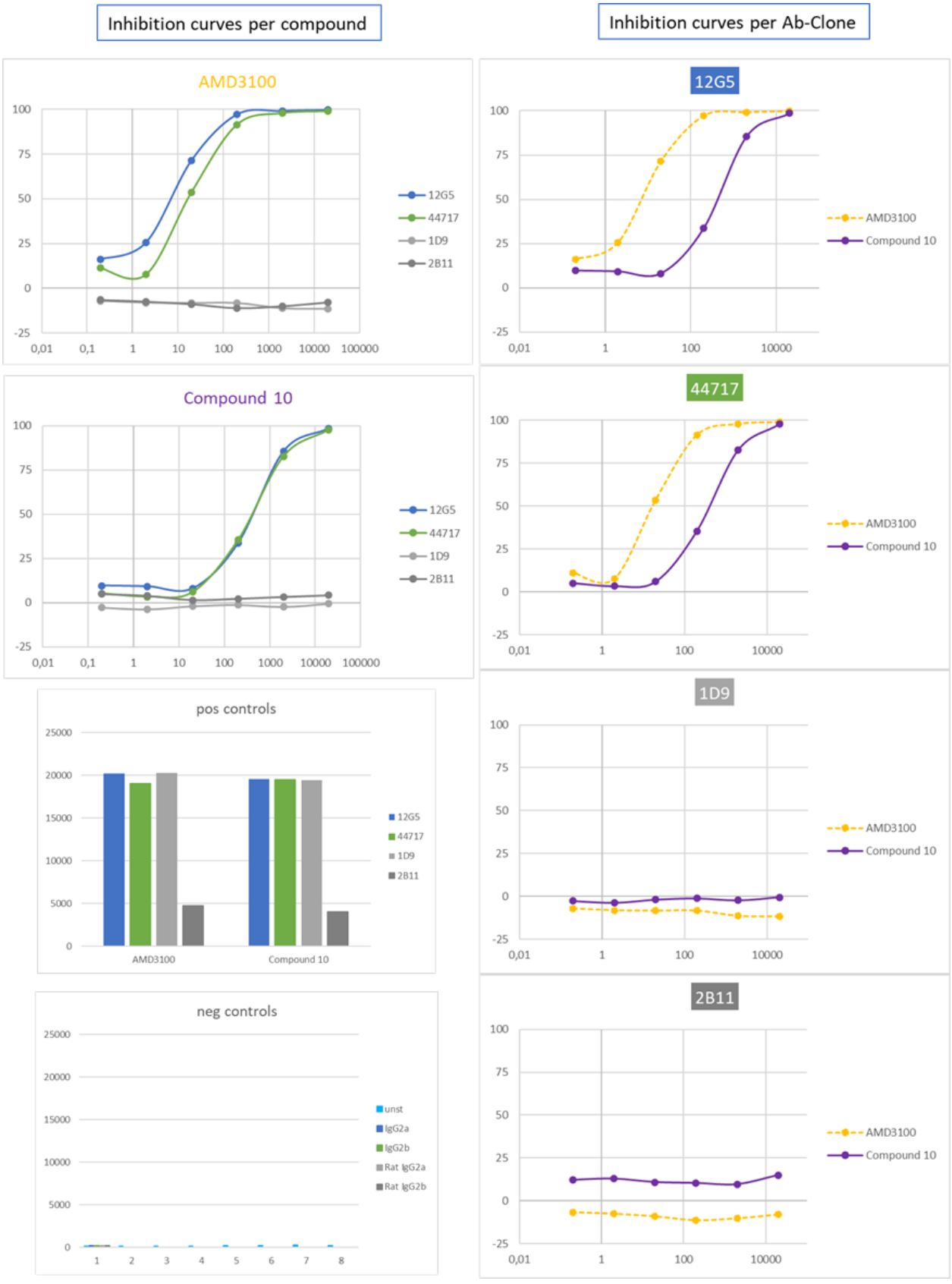


Table S4: *Inhibition of HIV-infection in TZM-bl, luciferase assay*

	NL4.3WT	NL4.3WT	BaL	BaL	Toxicity	Toxicity
	IC_{50} (µM)	**IC_{50} (µM)**	**IC_{50} (µM)**	**IC_{50} (µM)**	**CC_{50} (µM)**	**CC_{50} (µM)**
compound 1	**0,11**	**0,082**	**>20**	**>20**	**71,74**	**>100**
AMD3100	**0,00043**	**0,00031**	**-**	**-**	**>100**	