Electronic Supplementary Information (ESI) for:

Rigid H_4OCTAPA derivatives as model chelators for the development of Bi(III)-based radiopharmaceuticals

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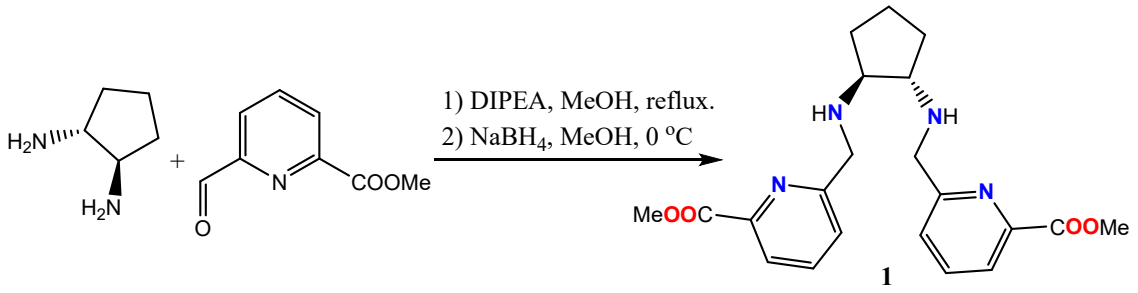
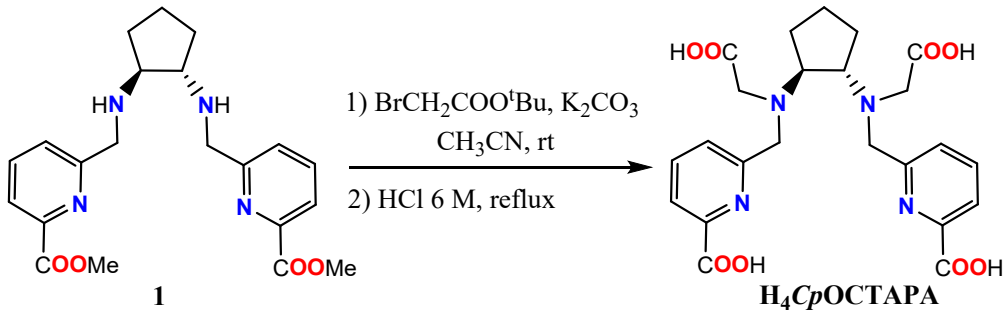
Content

Materials and methods. *trans*-Cyclopentane-1,2-diamine dihydrochloride was purchased from SpiroChem (Switzerland), methyl 6-formyl-2-pyridinecarboxylate and tert-butylbromoacetate were purchased from Fluorochem (United Kingdom), DIPEA was purchased from TCI (Japan) and sodium borohydride was purchased from AlfaAesar (United States). All products were used from providers, without further purifications. High-resolution electrospray-ionization time-of-flight ESI-TOF mass spectra were recorded in the positive and negative mode using a LTQ-Orbitrap Discovery Mass Spectrometers coupled to Thermo Accela HPLC. Medium performance liquid chromatography (MPLC) was carried out using a Puriflash XS 420 InterChim Chromatographer instrument equipped with a UV-DAD detector in reverse phase. Aqueous solutions were lyophilized using a Biobase BK-FD10 Series apparatus. ^{1}H and ^{13}C NMR spectra of the ligands and their precursors were on a Bruker AVANCE III 300, a Bruker AVANCE 400 or a Bruker AVANCE 500 spectrometers.



Scheme S1: Synthesis of **1**.

6,6-((1S,2S)-cyclopentane-1,2-diylbis(azanediyl))bis-(methylene))dipicolinic acid (1): 1) A solution of Methyl-6-formylpyridine-2-carboxylate (223.3 mg, 1.35 mmol) in MeOH (30 mL) was added dropwise to a refluxing solution of *trans*-1,2-cyclopentanediamine dihydrochloride (115.9 mg, 0.67 mmol) and N,N-Diisopropylethylamine (DIPEA, 0.23 mL, 1.34 mmol) in MeOH (10 mL). The resulting mixture was refluxed for 4 h. After this time, it was cooled to 0 ^{0}C and $NaBH_4$ (36.6 mg, 0.97 mmol) was added. The mixture was stirred at 0 ^{0}C for additional 1.5 h, until complete reduction of the imine confirmed by MS. Then saturated $NaHCO_3$ aqueous solution (50 mL) was added and it was stirred for 10 min. The resulting solution was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to give an orange oil (227.9 mg, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (dd, J = 7.7, 1.1 Hz, 2H), 7.60 (t, J = 7.7 Hz, 2H), 7.44 (dd, J = 7.8, 1.1 Hz, 2H), 3.86 (s, 2H), 3.82 (s, 2H), 3.75 (s, 6H), 2.67 (td, J = 5.1, 2.6 Hz, 2H), 1.79 (tt, J = 12.7, 6.5 Hz, 2H), 1.47 (p, J = 7.3, 6.9 Hz, 2H), 1.19 (dq, J = 12.8, 7.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.62, 160.94, 147.17, 137.24, 125.66, 123.28, 64.82, 53.52, 52.60, 31.14, 21.28, 19.98. MS (ESI^+, MeOH/H_2O): m/z 399.1702; calculated for $[C_{21}H_{26}N_4O_4]H^+$ 399.2027.



Scheme S2: Synthesis of **H_4*Cp*OCTAPA.**

N,N'-Bis(6-carboxy-2-pyridylmethyl)*trans*-1,2-cyclopentanediamine-N,N'-diacetic acid (H_4*Cp*OCTAPA): the oil **1** (271.6 mg, 0.67 mmol) was dissolved in CH_3CN (50 mL) and Na_2CO_3 (766.1 mg, 5.54 mmol) and tert-butylbromoacetate (0.27 mL, 1.84 mmol) were added. The mixture was stirred for 11 days, and then the excess Na_2CO_3 was filtered off. The filtrate was evaporated to dryness and the residue is redisolved in H_2O and extracted with DCM (4 x 25 mL). The organic phases were combined together and dried over Na_2SO_4, filtered, and evaporated to dryness to give an orange oil (415.3 mg). Finally, the oil is treated with 20 mL of HCl 6M at reflux overnight. The product was lyophilized to afford a yellow solid

that was purified by MPLC on reverser phase using a C18AQ (20 g) column and H_2O (0.1 % TFA) / CH_3CN (0.1 % TFA) as mobile phase (compound eluted at 42 % CH_3CN). Yellow solid (206.1 mg, 43% yield). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.27 (t, *J* = 7.8 Hz, 1H), 8.15 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.87 (dd, *J* = 7.9, 1.1 Hz, 2H), 4.60 (d, *J* = 2.4 Hz, 2H), 4.08 (d, *J* = 6.3 Hz, 1H), 3.84 (t, *J* = 1.3 Hz, 2H), 2.35 – 2.20 (m, 1H), 2.02 – 1.80 (m, 2H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 172.29, 165.23, 151.57, 146.09, 143.23, 128.05, 125.38, 65.89, 55.80, 52.78, 23.96, 20.44. Elemental analysis calcd (%) for $C_{23}H_{26}N_4O_8$ ·2TFA: C 45.39, H 3.95, N 7.84; found: C 45.91, H 4.12, N 7.67. IR (ATR, cm^{-1}): 1713 and 1620 ν(C=O). MS (ESI^+, MeOH/H_2O): m/z 509.1643; calculated for $[C_{23}H_{26}N_4O_4]Na^+$ 509.1643.

General synthesis procedure for the bismuth complexes: $Bi(NO_3)_3$ is added to a solution of H_4L in water in a molar ratio of 1:1.1 (H_4L: $Bi(NO_3)_3$) (5 mL). The mixture is left stirring at room temperature during 30 minutes. After this time, pH is adjusted to *ca* 4 with a solution of KOH (1M). Then, the precipitate is filtrated and the water solution was lyophilized. The crude was purified by MPLC on reverser phase using a C18AQ (20 g) column and H_2O (0.1 % HCOOH) / CH_3CN (0.1 % HCOOH) as mobile phase.

[BiOCTAPA]$^-$ pale yellow solid (43.03, 44% yield). ^{1}H NMR (300 MHz, Deuterium Oxide) δ 8.23 (dd, *J* = 8.4, 7.0 Hz, 2H), 8.06 (m, 2H), 7.84 (m, 2H), 5.03 (d, *J* = 15.8 Hz, 1H), 4.38 (m, 2H), 4.00 (m, 3H), 3.72 (d, *J* = 11.2 Hz, 1H), 3.61 (d, *J* = 16.8 Hz, 1H), 3.46 (d, *J* = 11.4 Hz, 1H).* ^{13}C NMR (126 MHz, Deuterium Oxide) δ 180.47 (b), 178.55 (b), 170.78 (b), 155.47 (b), 149.32 (b), 142.15 (b), 141.82, 141.47, 127.43 (b), 126.93, 125.82, 125.60, 63.14, 60.07(b), 59.22(b), 58.11(b), 56.25, 53.12, 52.41, 50.11, 49.63. IR (ATR, cm^{-1}): 1682 and 1588 ν(C=O). MS (ESI^-, MeOH/H_2O): m/z 651.0937; calculated for $[Bi(C_{20}H_{18}N_4O_4)]^-$ 651.0934.

*The assignment of the ^{1}H NMR is not accurate due to the presence of a second species in solution in combination with the fluxionality of the system.

[Bi(H*CHX*OCTAPA)] White solid (43% yield). ^{1}H NMR (300 MHz, Deuterium Oxide) δ 8.27 (m, 2H), 8.08 (dd, *J* = 22.0, 7.8 Hz, 2H), 7.88 (d, *J* = 7.7 Hz, 2H), 5.42 (d, *J* = 14.9 Hz, 1H), 4.20 (d, *J* = 15.6 Hz, 1H), 3.99 (d, *J* = 15.0 Hz, 1H), 3.49 m, 2H), 3.03 (t, *J* = 10.8 Hz, 1H), 2.36 (m, 2H), 1.80 (m, 3H), 1.47 (m, 1H), 1.23 (m, 2H), 0.83 (m, 1H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 181.94, 179.65, 171.45, 170.08, 156.02, 152.77, 150.62, 148.77, 141.82 (d, *J* = 8.0 Hz), 127.27, 126.08 (d, *J* = 5.2 Hz), 125.27, 68.06, 65.44, 63.32, 61.21, 54.99, 53.66, 27.86, 25.52, 24.67, 23.47. IR (ATR, cm^{-1}): 1620 and 1581 ν(C=O). MS (ESI^- MeOH/H_2O): m/z 705.1406; calculated for $[Bi(C_{24}H_{24}N_4O_4)]^-$ 705.1403.

[Bi(H*Cp*OCTAPA)] yellow solid (27.9 mg, 40% yield). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.16 (t, *J* = 7.8 Hz, 1H), 8.06 (t, *J* = 7.7 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.71 (t, *J* = 8.6 Hz, 2H), 5.19 (d, *J* = 15.2 Hz, 1H), 4.73 (d, *J* = 15.9 Hz, 1H), 4.50 (d, *J* = 18.6 Hz, 1H), 4.06 (d, *J* = 15.8 Hz, 1H), 3.96 (d, *J* = 15.2 Hz, 1H), 3.41 (d, *J* = 16.4 Hz, 1H), 3.16 (d, *J* = 18.6 Hz, 1H), 3.01 (m, 1H), 2.79 (q, *J* = 10.1, 9.5 Hz, 1H), 1.87 (m, 2H), 1.54 (m, 2H), 1.32 (m, 1H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 182.34, 180.37, 171.59, 170.30, 156.23, 153.38, 150.54, 148.76, 141.88, 141.56, 127.19, 126.01, 125.84, 125.35, 69.18, 64.39, 62.40, 56.17, 52.52, 21.50, 19.31, 19.14. IR (ATR, cm^{-1}): 1679 and 1575 ν(C=O). MS (ESI^-, MeOH/H_2O): m/z 691.1253; calculated for $[Bi(C_{23}H_{22}N_4O_4)]^-$ 691.1247.

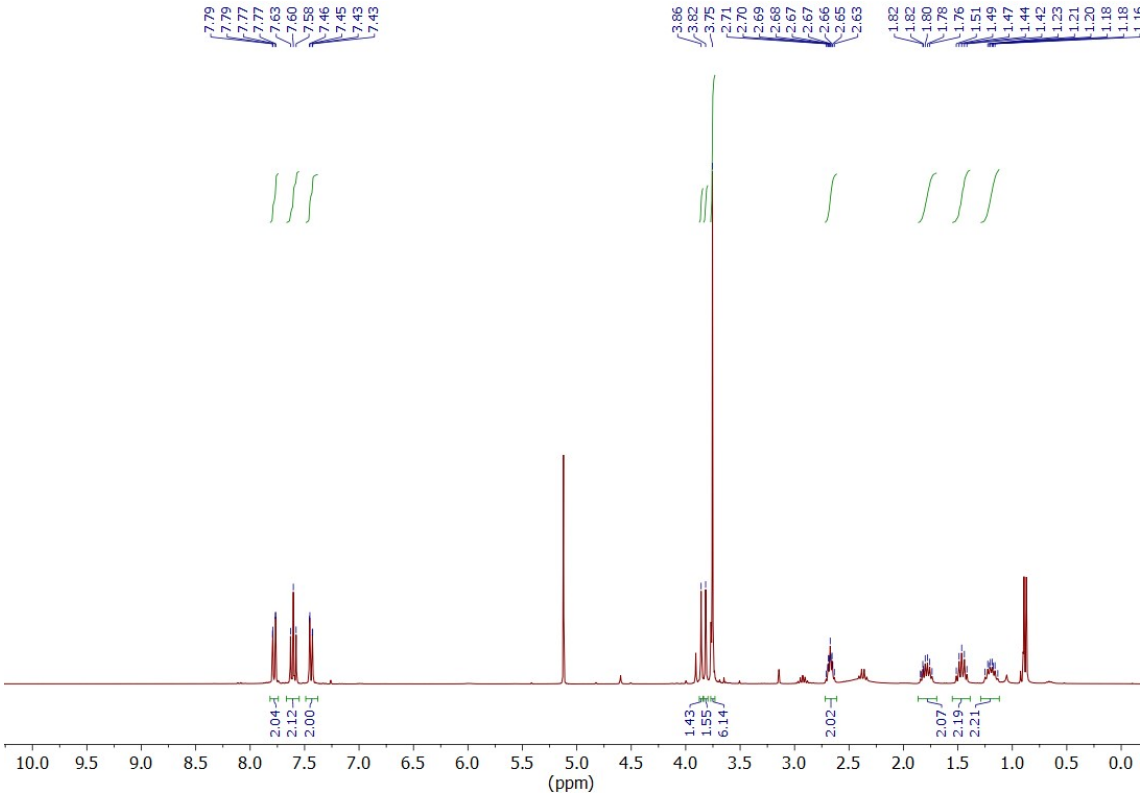


Figure S1: ^{1}H NMR spectrum of compound **1** (300 MHz, $CDCl_3$, 298 K).

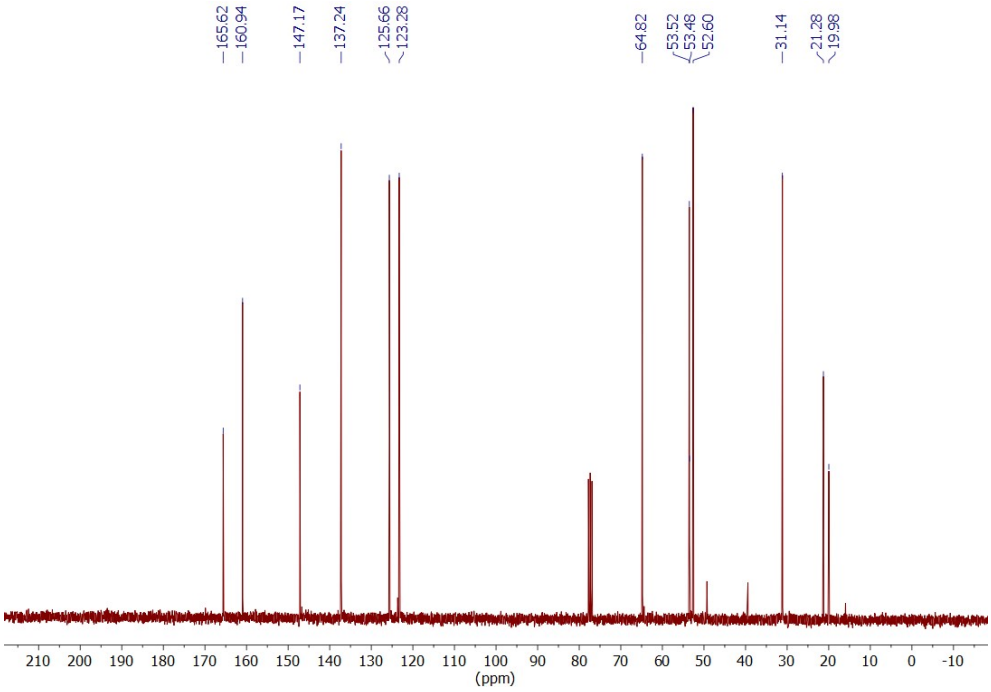


Figure S2: ^{13}C NMR spectrum of compound **1** (300 MHz, $CDCl_3$, 298 K).

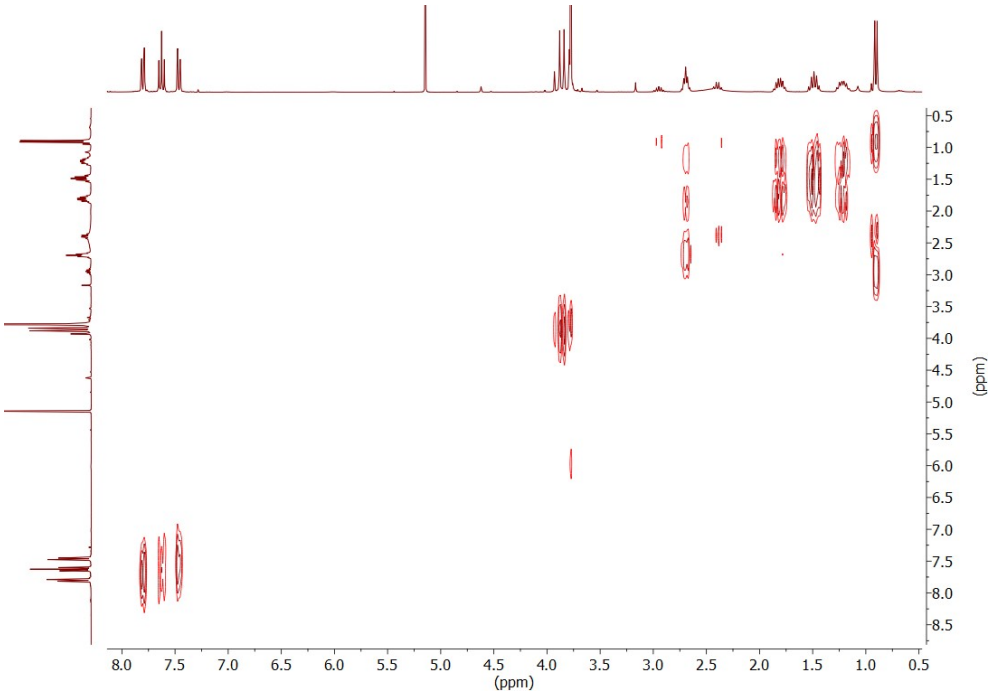


Figure S3: COSY spectrum of compound **1** (300 MHz, $CDCl_3$, 298 K).

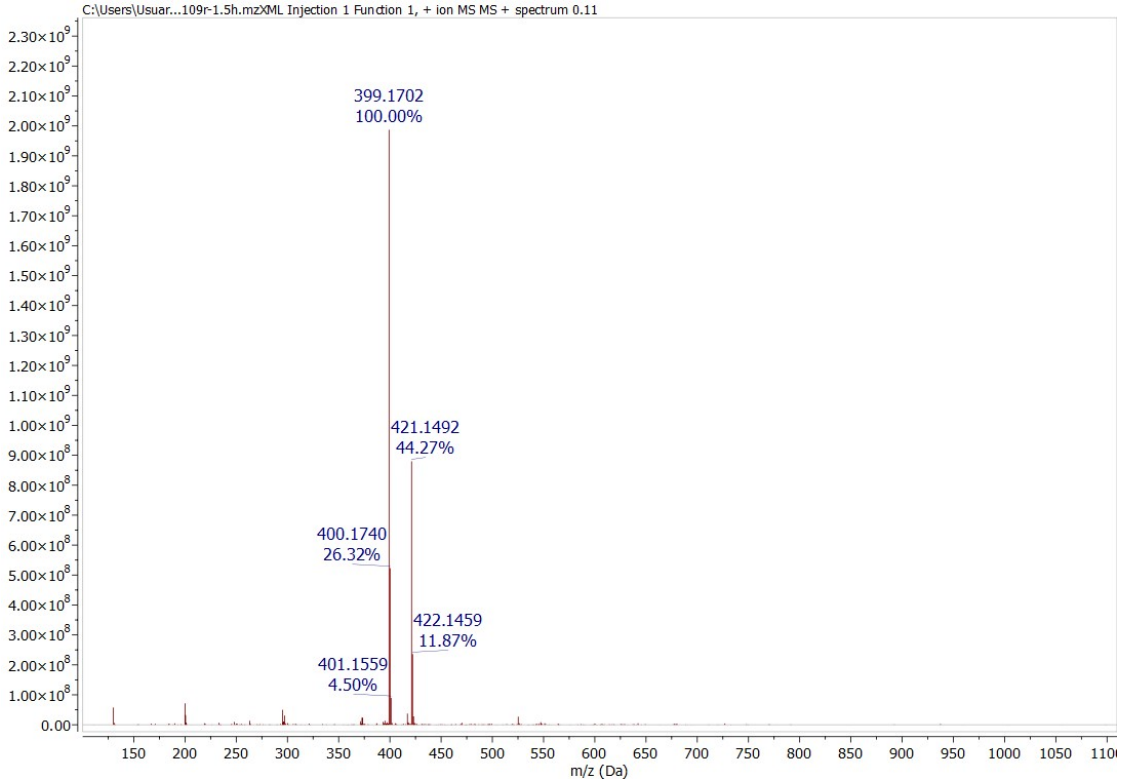


Figure S4: Experimental high resolution mass spectrum (ESI^+) of **1**.

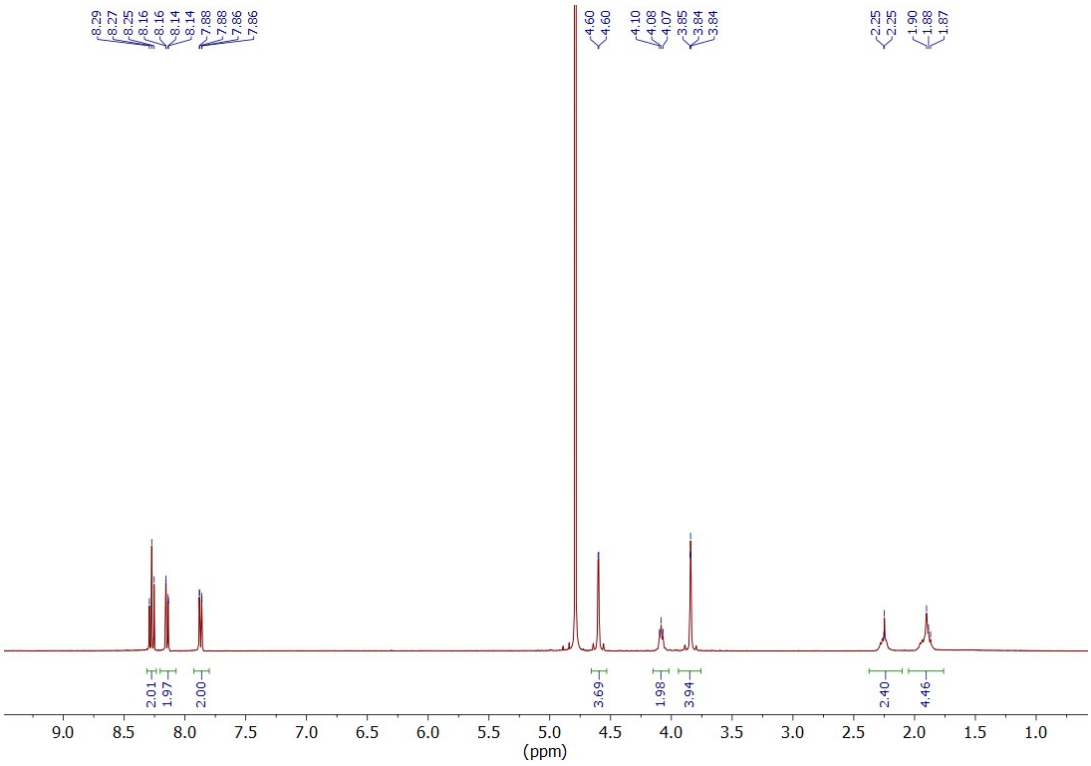


Figure S5: ^{1}H NMR spectrum of **H_4*Cp*OCTAPA** (400 MHz, D_2O, 298 K, pH 1).

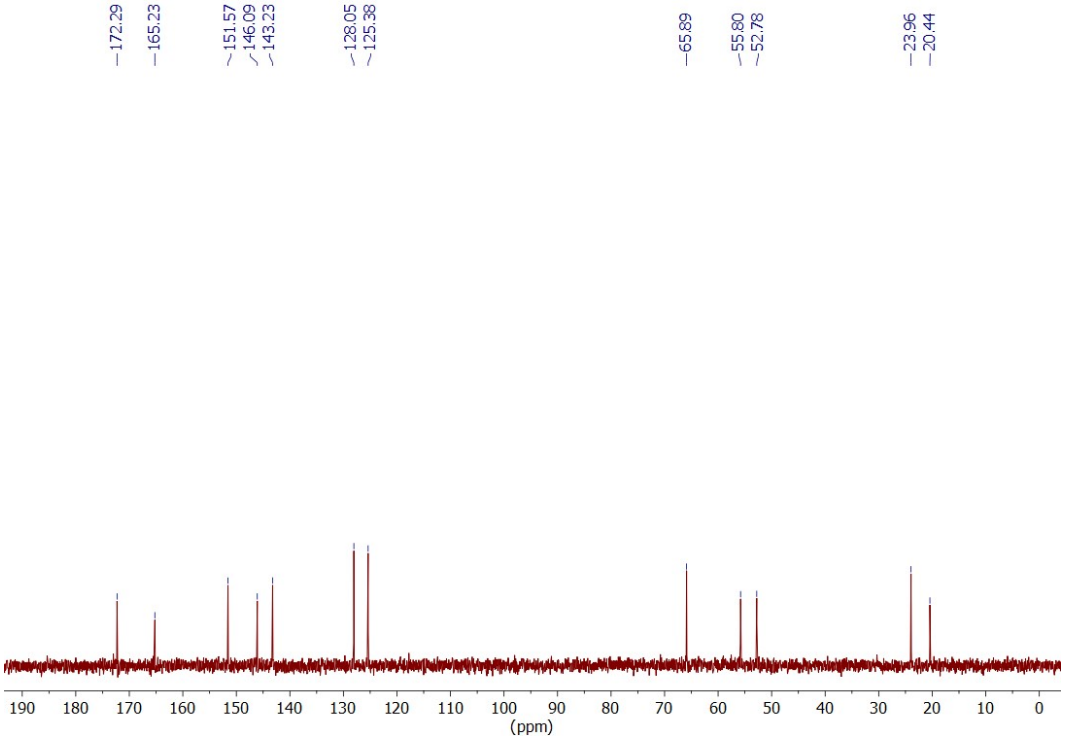


Figure S6: ^{13}C NMR spectrum of **H_4*Cp*OCTAPA** (101 MHz, D_2O, 298 K, pH 1).

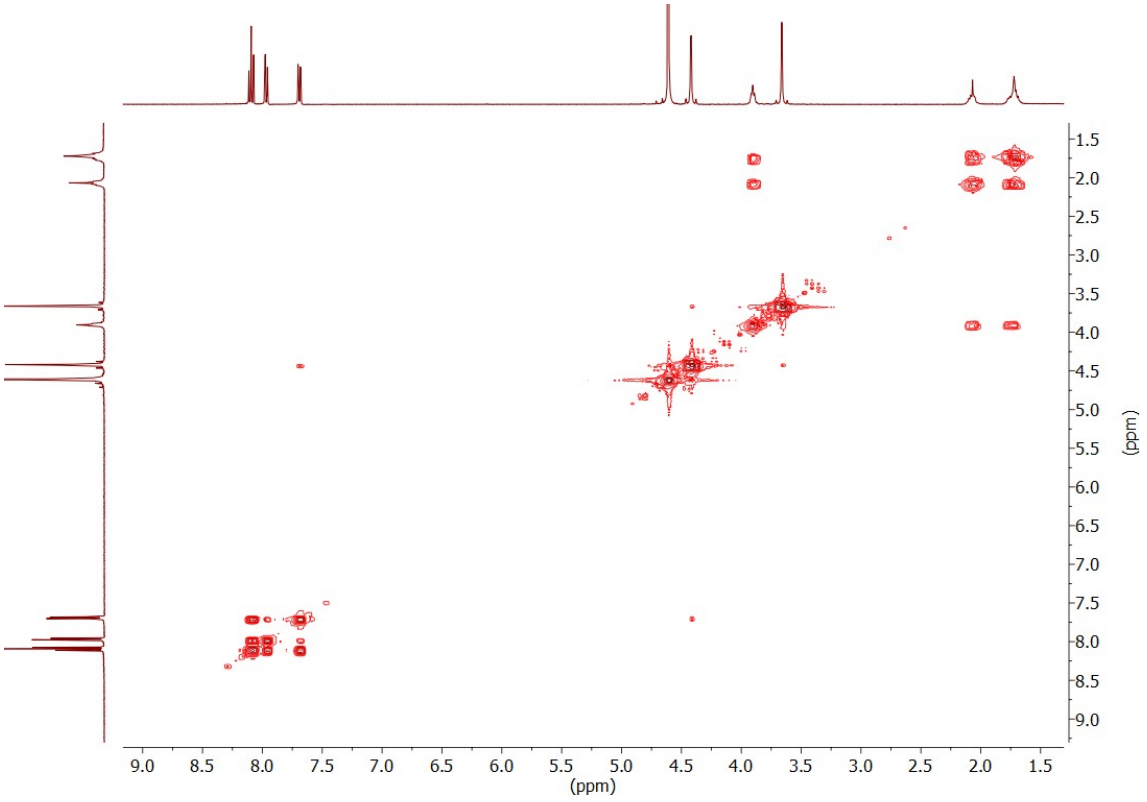


Figure S7: COSY spectrum of **H_4*Cp*OCTAPA** (400 MHz, D_2O, 298 K, pH 1).

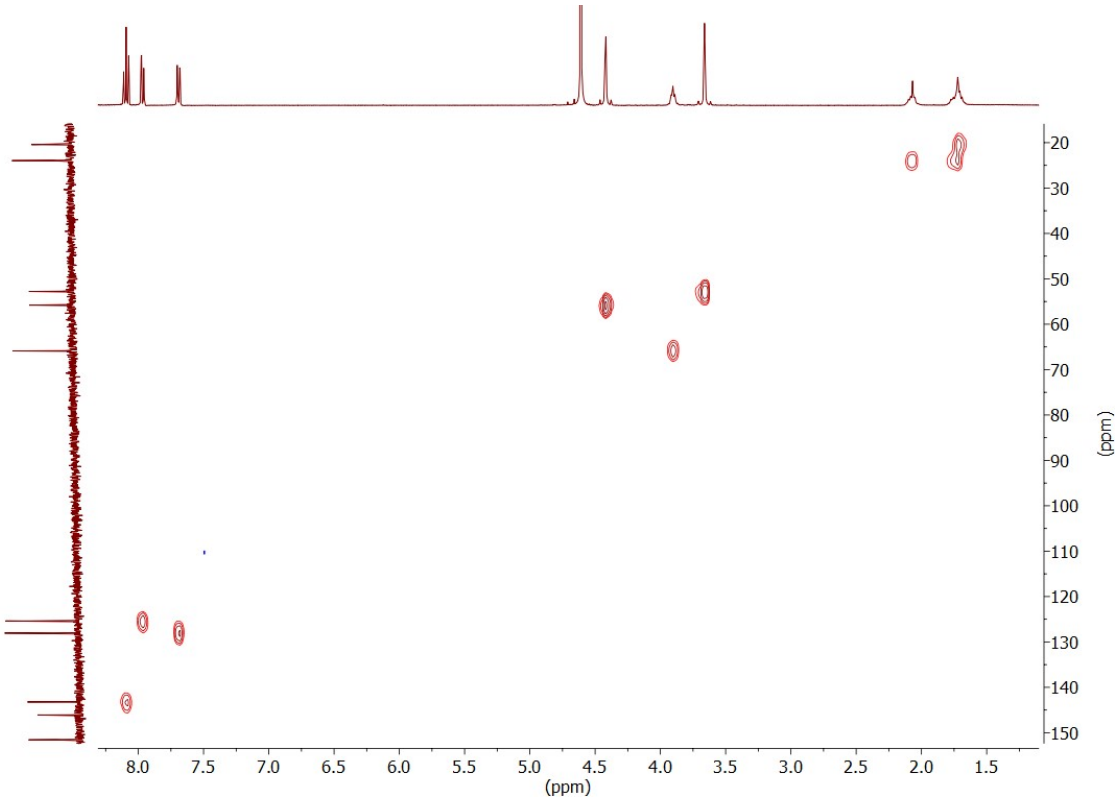


Figure S8: HSQC spectrum of **H_4*Cp*OCTAPA** (400 MHz, D_2O, 298 K, pH 1).

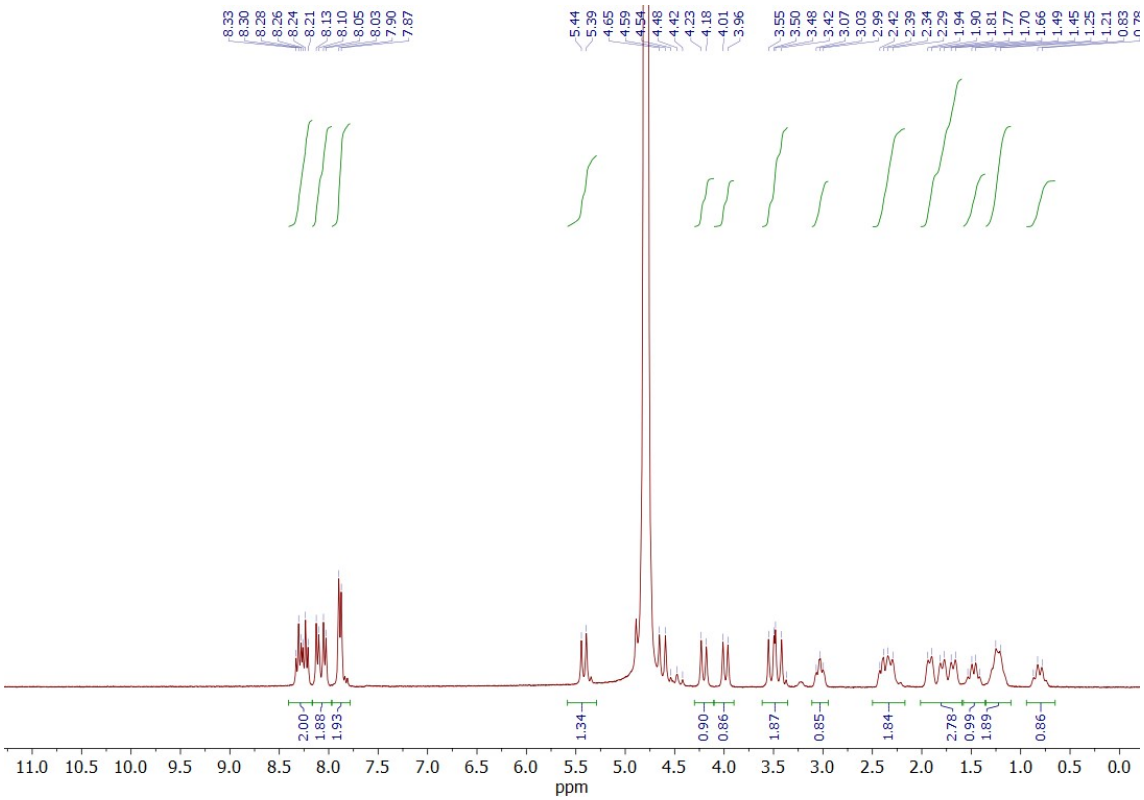


Figure S9: ^{1}H NMR spectrum of **Bi[*CHX*OCTAPA]**$^{-}$ (400 MHz, D_2O, 298 K, pH 5.0).

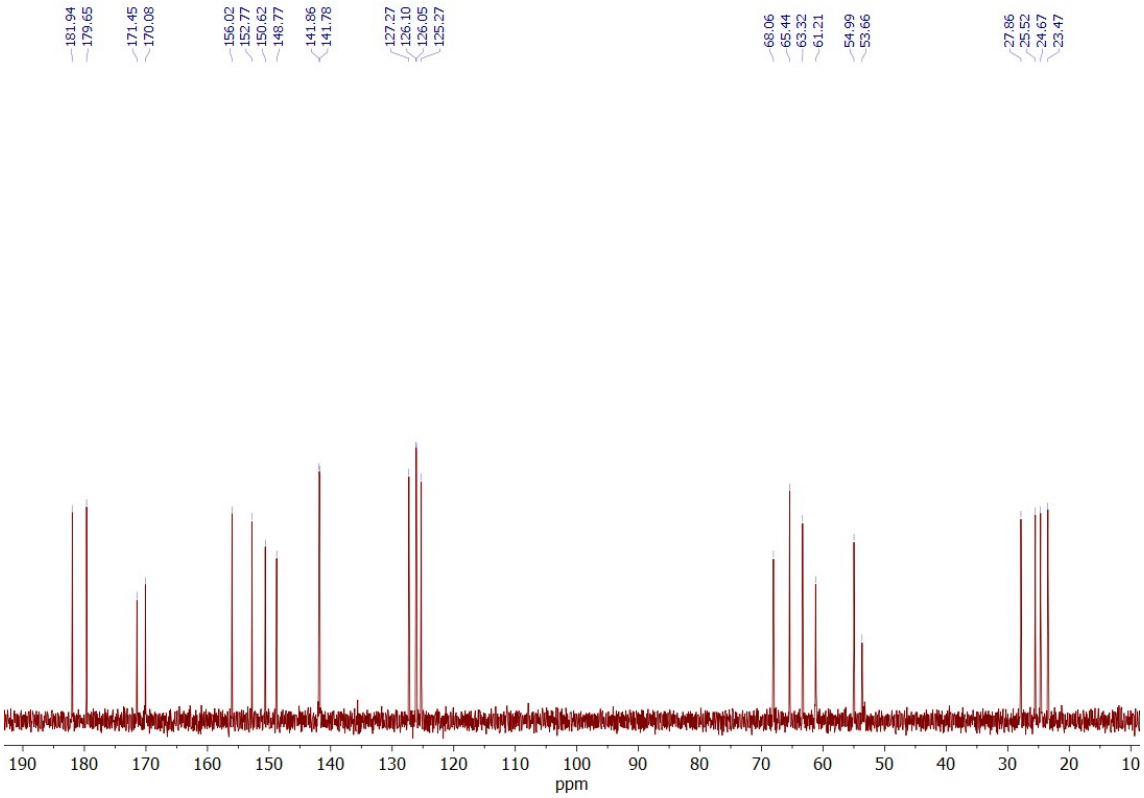


Figure S10: ^{13}C NMR spectrum of **Bi[*CHX*OCTAPA]**$^{-}$ (400 MHz, D_2O, 298 K, pH 5.0).

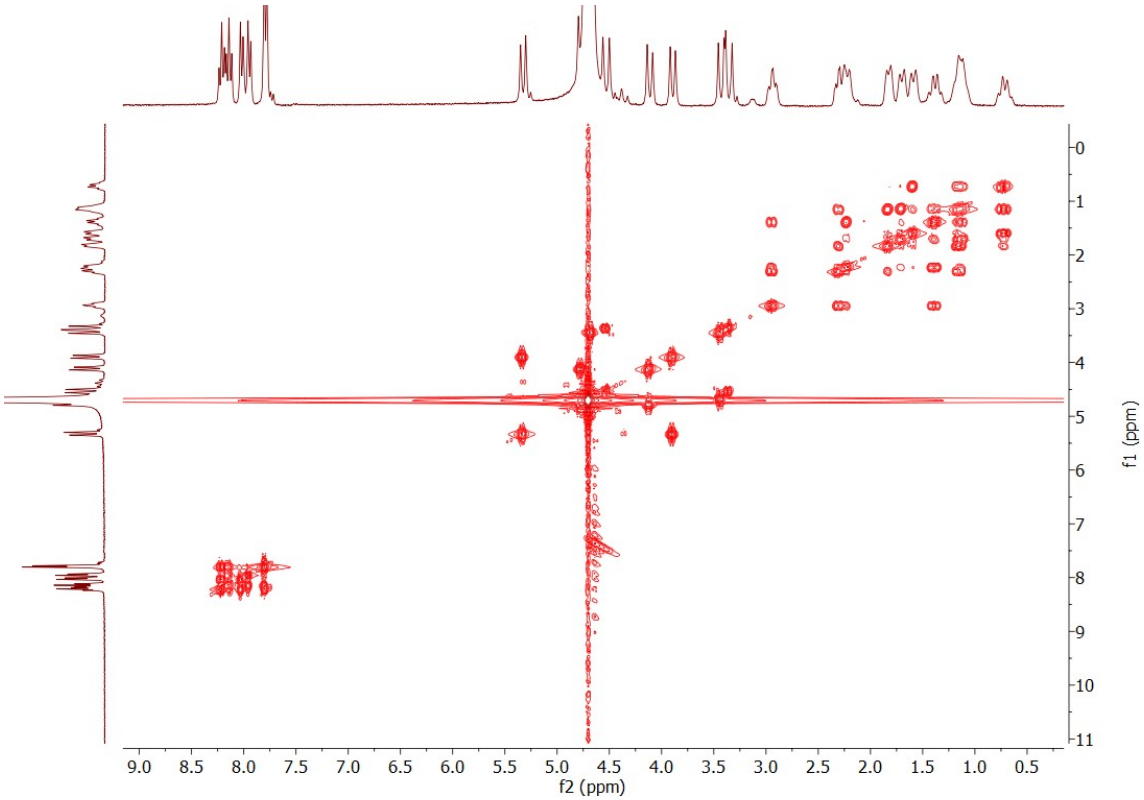


Figure S11: COSY spectrum of **Bi[*CHX*OCTAPA]**$^{-}$ (400 MHz, D_2O, 298 K, pH 5.0).

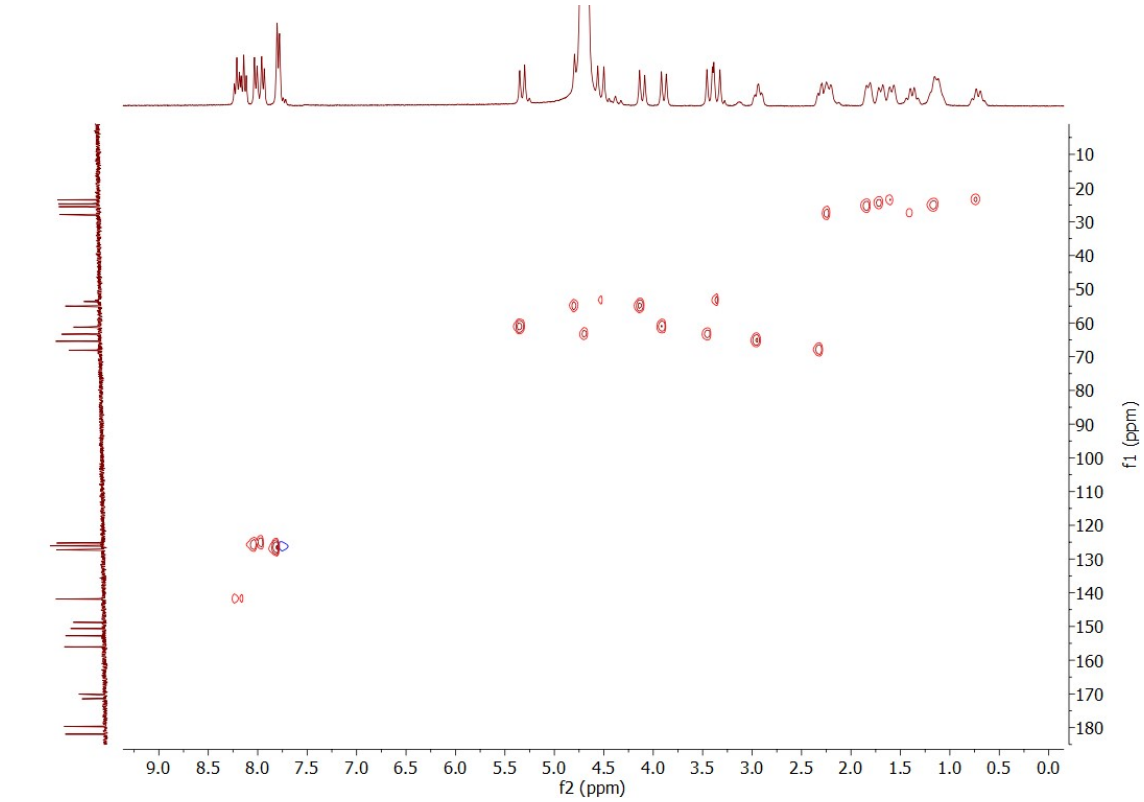


Figure S12: HSQC spectrum of **Bi[*CHX*OCTAPA]**$^{-}$ (400 MHz, D_2O, 298 K, pH 5.0).

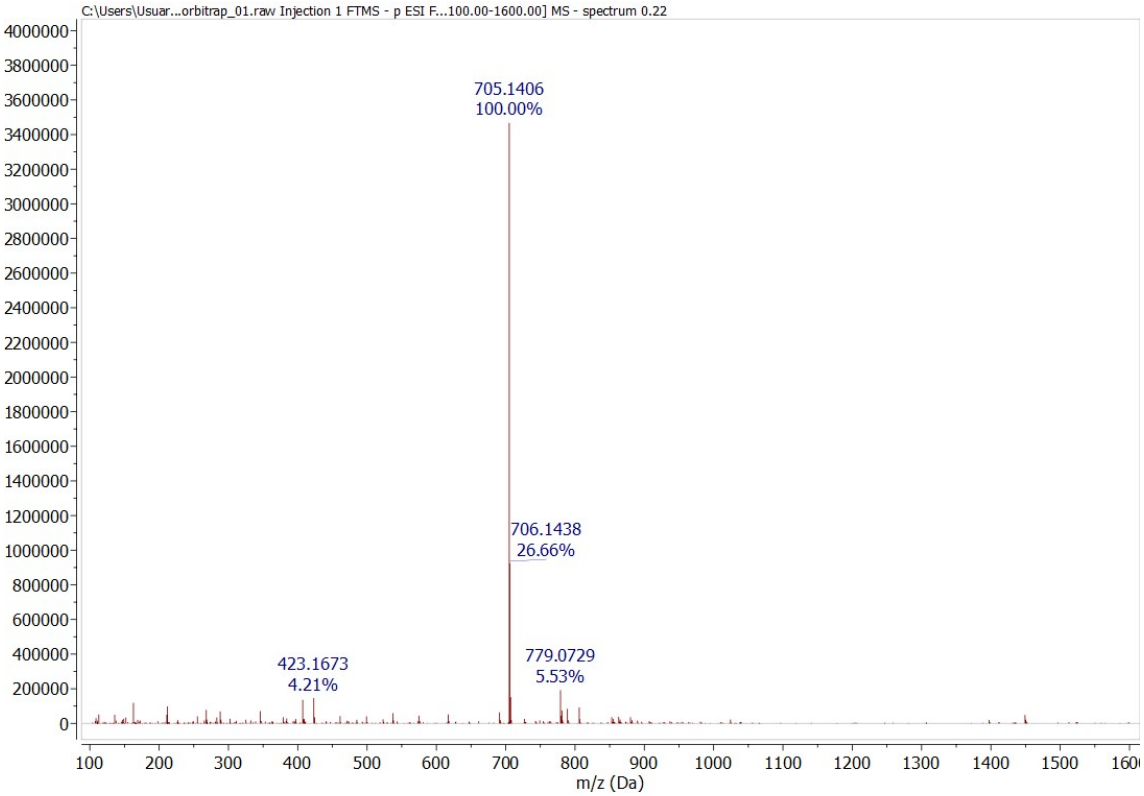


Figure S13: Experimental high resolution mass spectrum (ESI$^-$) of **Bi[*Cp*OCTAPA]**$^-$.

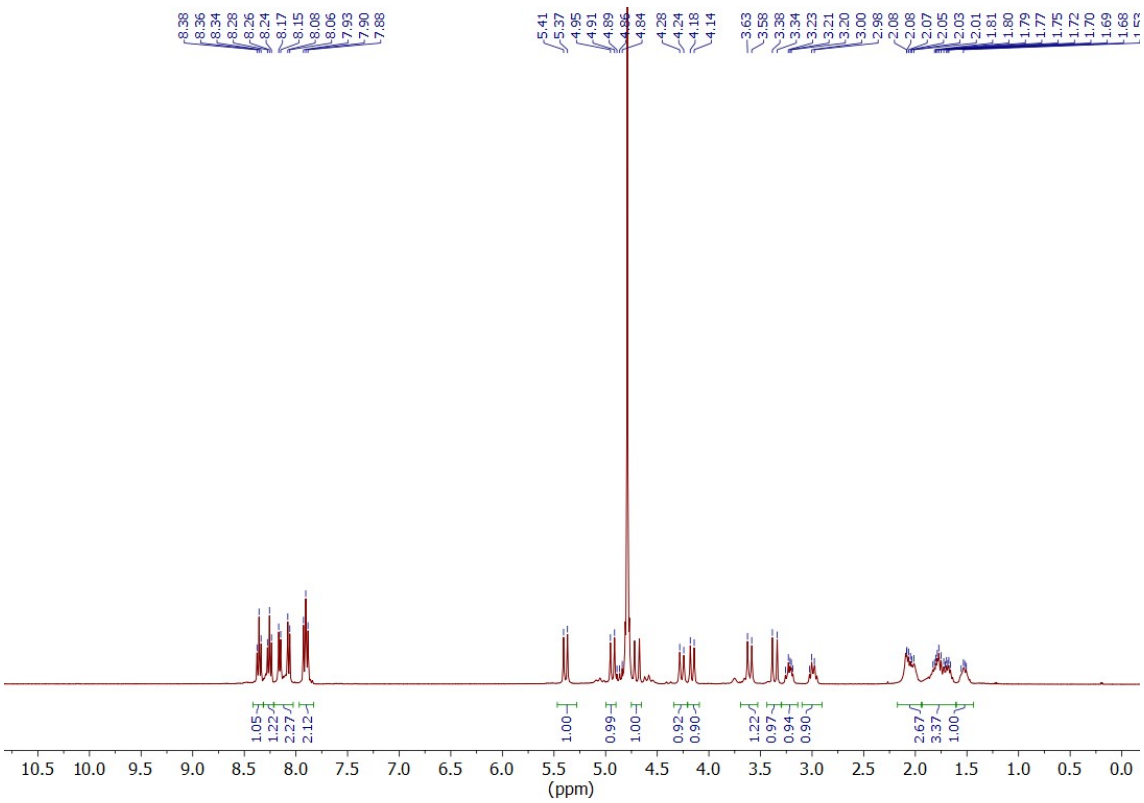


Figure S14: ^{1}H NMR spectrum of **Bi[*Cp*OCTAPA]**$^-$ (400 MHz, D_2O, 298 K, pH 2.0).

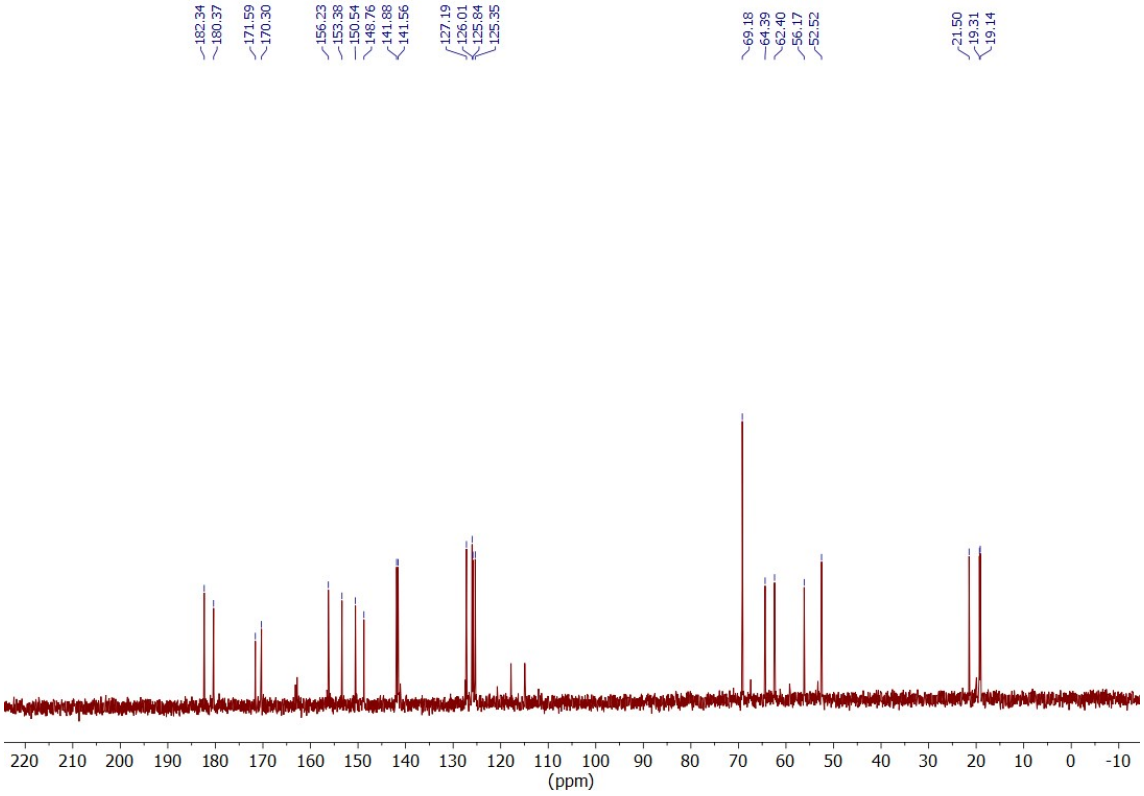


Figure S15: ^{13}C NMR spectrum of **Bi[*Cp*OCTAPA]**$^{-}$ (101 MHz, D_2O, 298 K, pH 2.0).

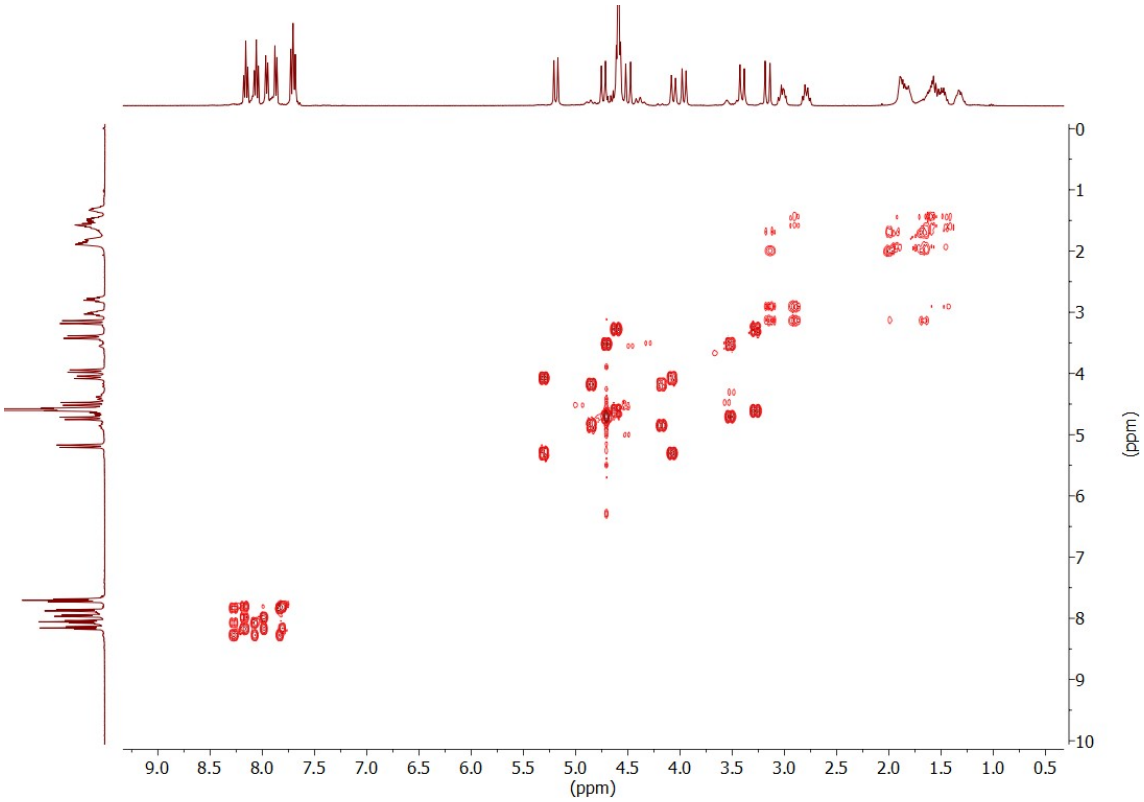


Figure S16: COSY spectrum of **Bi[*Cp*OCTAPA]**$^{-}$ (400 MHz, D_2O, 298 K, pH 2.0).

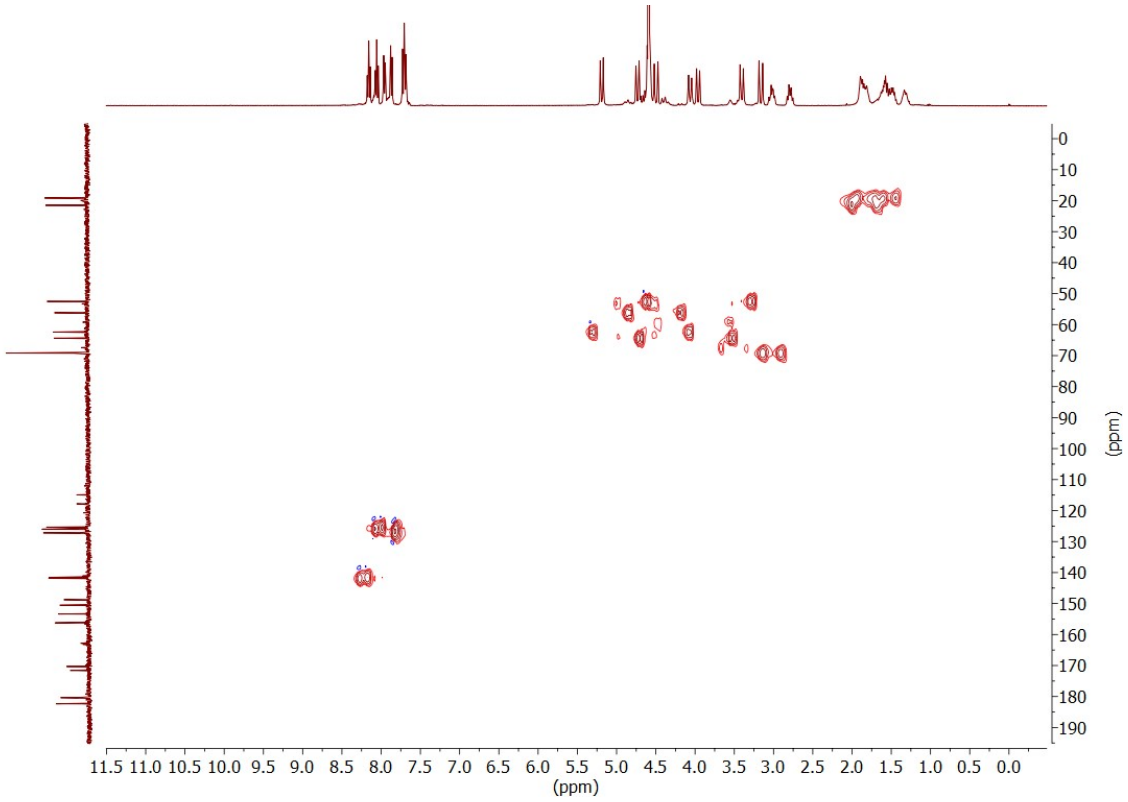


Figure S17: HSQC NMR spectrum of **Bi[*Cp*OCTAPA]**$^{-}$ (400 MHz, D_2O, 298 K, pH 2.0).

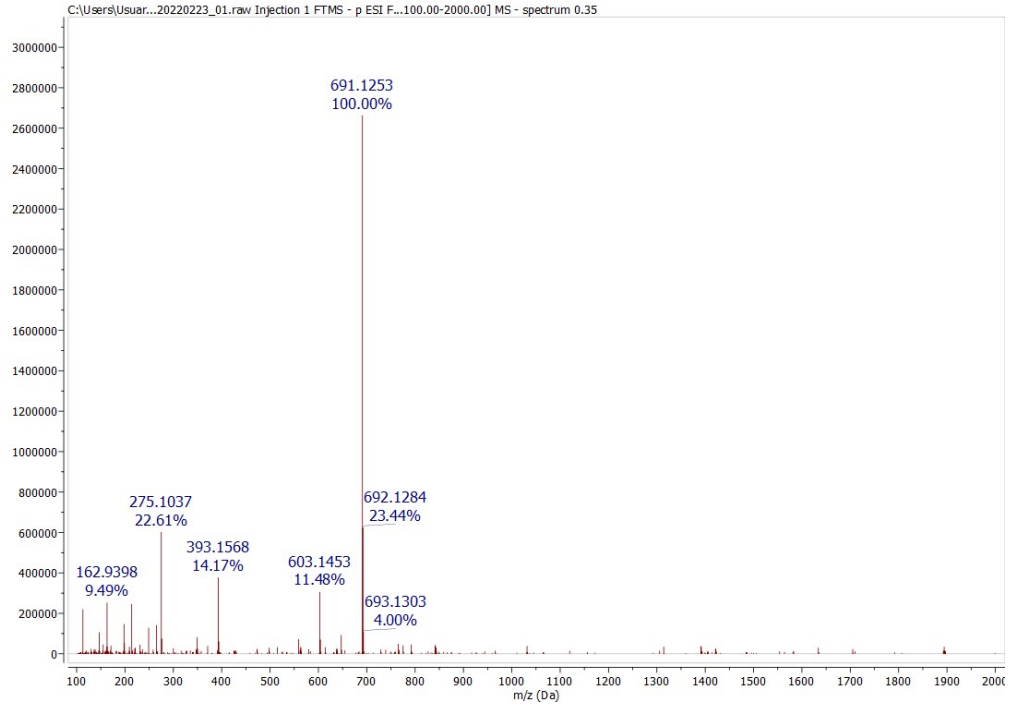


Figure S18: Experimental high resolution mass spectrum (ESI^{-}) of **Bi[*Cp*OCTAPA]**$^{-}$.

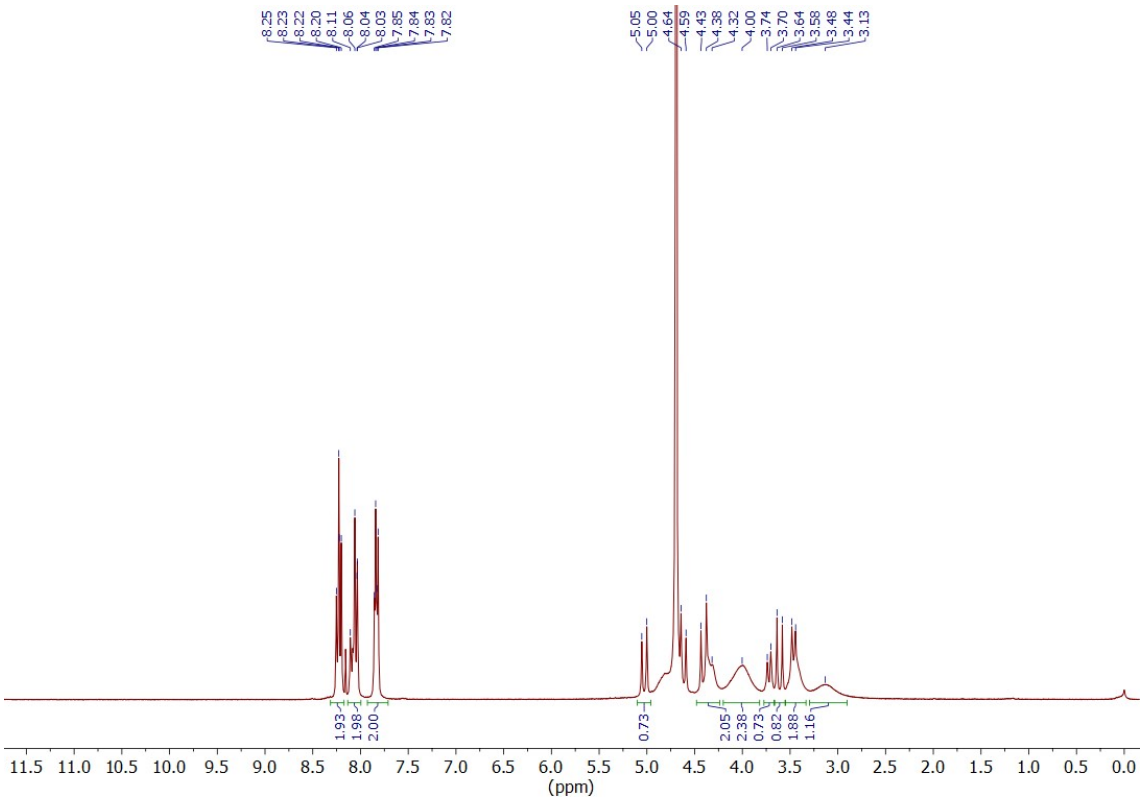


Figure S19: ^{1}H NMR spectrum of **Bi[OCTAPA]**$^{-}$ (300 MHz, D_2O, 298 K, pH 2.0).

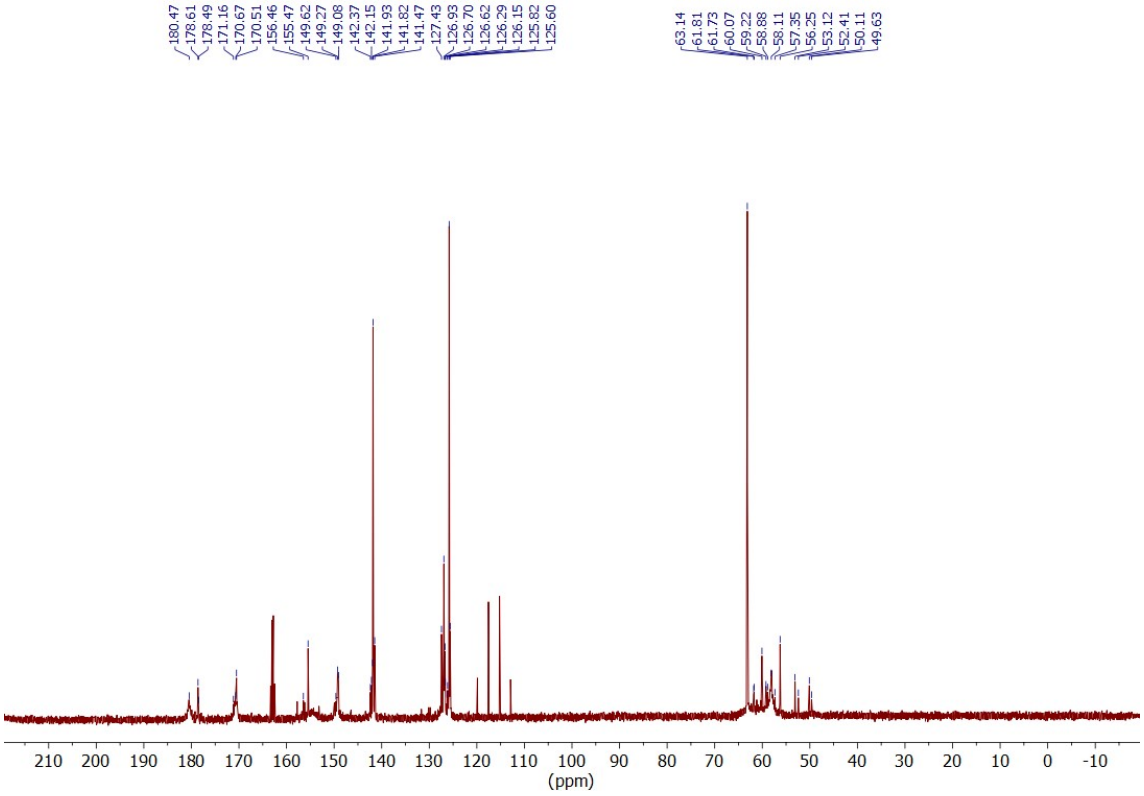


Figure S20: ^{1}H NMR spectrum of **Bi[OCTAPA]**$^{-}$ (500 MHz, D_2O, 298 K, pH 3.0).

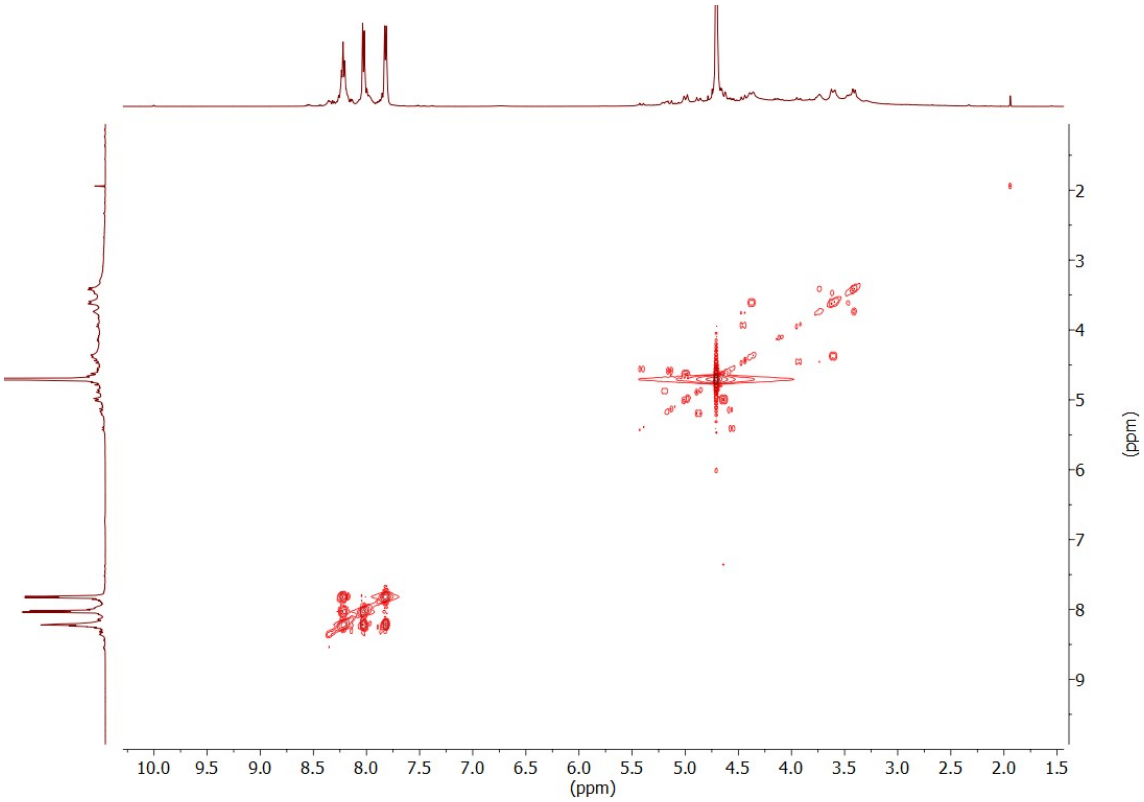


Figure S21: COSY spectrum of **Bi[OCTAPA]**$^{-}$ (500 MHz, D_2O, 298 K, pH 3.0).

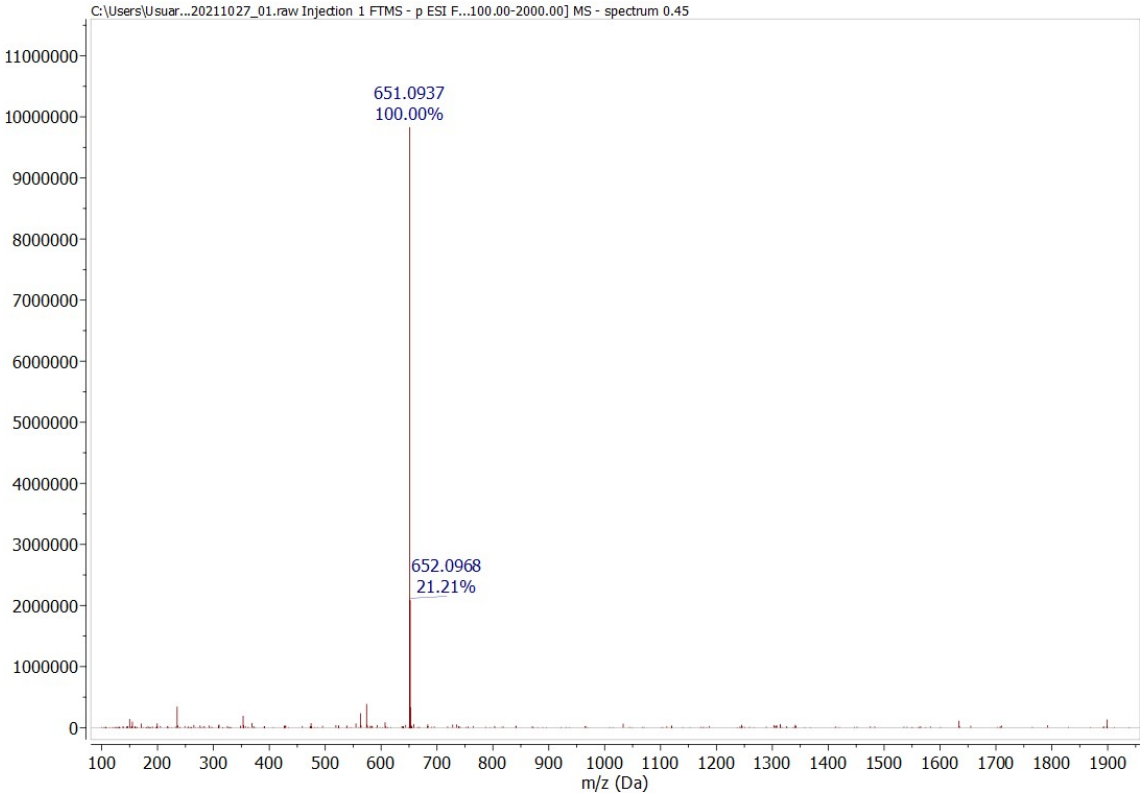


Figure S23: Experimental high resolution mass spectrum (ESI$^-$) of **Bi[*CHX*OCTAPA]$^-$**.

Stability constant determinations. The protonation constants of the ligand was determined by pH-potentiometric titrations with 0.15 M NaOH, using 0.0025 M ligand solutions. The ionic strength was set to 1.0 M by using NaBr. The titrated samples (starting volume of 5.00 mL) were stirred mechanically and thermostated at 25 °C by a circulating water bath (±0.1 °C). To avoid the effect of CO_2, N_2 gas was bubbled through the solutions during the titrations process. The pH-potentiometric titrations were performed with a Metrohm 785 DMP Titrino titration workstation with the use of a Metrohm 6.0234.100 combined electrode in the pH range of 1.75-11.75. For the calibration of the pH meter, KH-phtalate (pH = 4.005) and borax (pH = 9.177) buffers were used, and the H^+ ion concentrations were calculated from the measured pH values by applying the method of Irving et al..[i] The protonation constants of the ligand (log K_i^H) are defined as follows:

$$K_i^H = \frac{[H_i L]}{[H_{i-1} L][H^+]}$$

The stability of the Bi(III) complexes were investigated by monitoring the acid propagated dissociation of the complex in the presence of NaBr (I = 1 M NaBr), following the methodology reported in the literature by É. Csajbók et. al.. [ii] Samples containing Bi(III) at 6.89×10^{-5} M or 8.20×10^{-5} M (*Cp*OCTAPA) concentration and the ligands $c_{Lig}=5.02\times10^{-4}$ M or 5.90×10^{-4} M (*Cp*OCTAPA) were prepared and their acid concentration was varied in the range of 0.00357 – 0.536 M by tha addition of $HClO_4$. Spectra were recorded at 24, 18 and 17 different acid concentrations for the Bi(OCTAPA)$^-$, Bi(*CHX*OCTAPA)$^-$ and Bi(*Cp*OCTAPA)$^-$ complexes, respectively in wavelength range of 200-400 nm and stability constants were determined by using the absorbance data recorded at 360, 362, 364, 367, 371, 373, 377, and 380 nm. Protonation constants of the Bi(III) complexes were determined by titrating the preformed Bi(OCTAPA)$^-$, Bi(*CHX*OCTAPA)$^-$ and Bi(*Cp*OCTAPA)$^-$ complexes data in the presence of 1.0 M NaBr in the pH-range of 1.75-10.50. For the calculation of the equilibrium constants, the PSEQUAD program was used.[iii]

Table S1. Protonation constants of the **OCTAPA^{4-}**, ***CHX*OCTAPA^{4-}** and ***Cp*OCTAPA^{4-}** ligands and equilibrium constants characterizing the formation of their Bi(III) complexes (1M NaBr, 25 °C).

	OCTAPA^{4-}	***CHX*OCTAPA^{4-}**	***Cp*OCTAPA^{4-}**
log K_1^H	**8.39(2)**	**9.48(3)**	**8.18(5)**
log K_2^H	**5.21(3)**	**6.13(5)**	**5.87(5)**
log K_3^H	**3.49(3)**	**4.08(5)**	**3.52(6)**
log K_4^H	**2.78(4)**	**3.56(5)**	**2.69(5)**
log K_5^H	–	**1.07(8)**	–
Σ log K_4^H	**19.87**	**23.25**	**20.26**
log K_{BiL}	27.33(4)	30.63(6)	27.61(4)
logβ_{BiHL}	30.37(2)	33.20(2)	30.10(1)
log K_{BiHL}	3.04(2)	2.57(2)	2.49(5)
log $K_{BiL(OH)}$	9.95(2)		

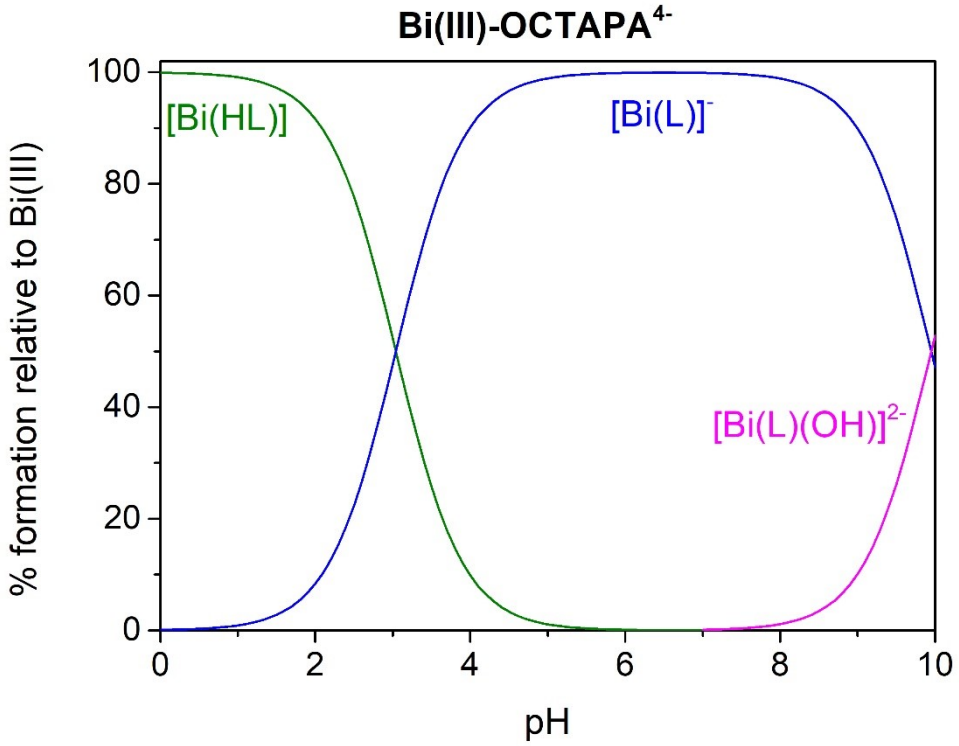


Figure S24: Species distribution curves calculated for the Bi(III):OCTAPA^{4-}:H$^+$ system (c_{Lig}=$c_{Bi(III)}$=10^{-3} M).

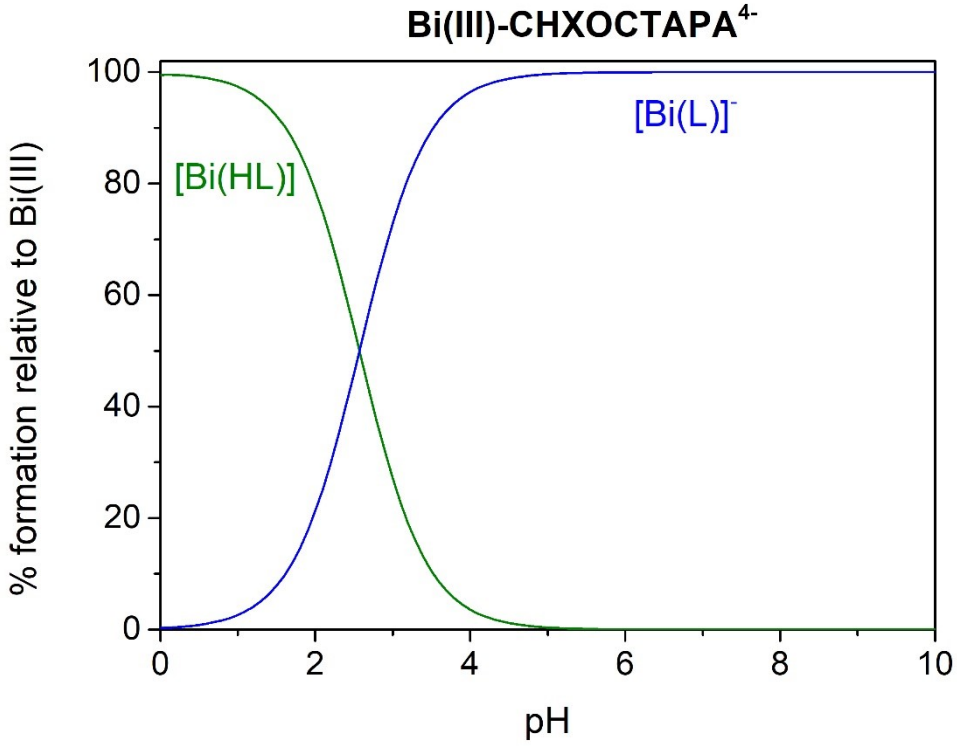


Figure S25: Species distribution curves calculated for the Bi(III):*CHX*OCTAPA^{4-}:H^{+} system (c_{Lig}=$c_{Bi(III)}$=10^{-3} M).

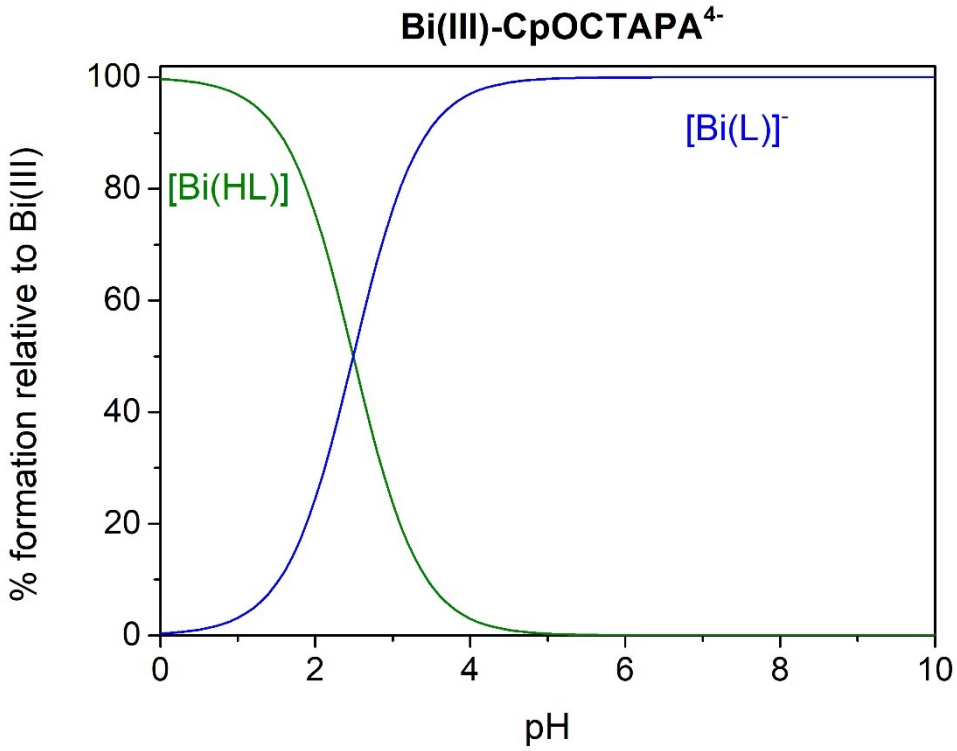


Figure S26: Species distribution curves calculated for the Bi(III):*Cp*OCTAPA^{4-}:H^{+} system (c_{Lig}=$c_{Bi(III)}$=10^{-3} M).

Table S2: Crystal data and structure refinement for **[Bi(H*Cp*OCTAPA)]** and **[Bi(H*CHX*OCTAPA)]**.

	[Bi(H*Cp*OCTAPA)]	**[Bi(H*CHX*OCTAPA)]**
Empirical formula	$C_{23}H_{33}BiN_4O_{13}$	$C_{24}H_{27}BiN_4O_9$
Molecular weight MW	782.51	724.47
Crystal system	Triclinic	Orthorhombic
Space group	P-1	$P2_12_12_1$
a/Å	10.0759(7)	8.9617(3)
b/Å	11.0409(7)	13.5542(5)
c/Å	12.3655(7)	20.2007(7)
α/°	102.261(2)	90
β/°	95.473(2)	90
γ/°	92.621(2)	90
Volume (Å^3)	1335.04(15)	2453.75(15)
Z	2	4
ρ_{calc} (g/cm^3)	1.947	1.961
M (mm^{-1})	6.679	7.248
θ range	2.04°-28.30°	2.02°-28.32°
R_{int}	0.0389	0.0349
Measured reflections	65778	31991
Independent reflections / unique (I >2σ (I)	6647 / 6562	6097 / 5907
Goodness-of-fit on F^2	1.085	1.057
R1	0.0119	0.0238
wR2 (all data)	0.0286	0.0529
Larg. Diff. peak and hole (eÅ^{-3})	0.53 and -0.91	2.36 and -0.95
Flack parameter		-0.027(3)

Crystal structure determinations. Single crystals of **[Bi(H*Cp*OCTAPA)].5H$_2$O** and **[Bi(H*CHX*OCTAPA)(H$_2$O)]** were analysed by X-ray diffraction. **Table S1** shows the crystallographic data and the structure refinement parameters. Crystallographic data were collected at 100 K using a **Bruker D8 Venture diffractometer with a Photon 100 CMOS detector and Mo-Kα radiation** (λ = 0.71073 Å) generated by an Incoatec high brillance microfocus source equipped with Incoatec Helios multilayer optics. APEX3[iv] software was used for collecting frames of data, indexing reflections, and the determination of lattice parameters, while SAINT[v] was used for integration of intensity of reflections, and SADABS[vi] for scaling and empirical absorption correction. The structure was solved by dual-space methods using the program SHELXT.[vii] All non-hydrogen atoms were refined with anisotropic thermal parameters by full-matrix least-squares calculations on F^2 using the program SHELXL-2014.[viii] Hydrogen atoms positions were calculated and constrained with isotropic thermal parameters. For **[Bi(H*CHX*OCTAPA)(H$_2$O)]**, highly disordered solvent molecules were removed using the Solvent Mask routine from OLEX 2.[90] [ix] CCDC 2218896 and 2218897 contains the supplementary crystallographic data for **[Bi(H*Cp*OCTAPA)].5H$_2$O** and **[Bi(H*CHX*OCTAPA)(H$_2$O)]** respectively. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.ac.uk/data_request/cif.

Table S3. Bond distances (Å) of the metal coordination environment in crystals of **[Bi(H*CHX*OCTAPA)(H$_2$O)]** and **[Bi(H*Cp*OCTAPA)]·5H$_2$O**.

Ligand	**H$_4$*CHX*OCTAPA**	**H$_4$*Cp*OCTAPA**
Bi(1)-N(1)	2.701(4)	2.5993(13)
Bi(1)-N(2)	2.674(4)	2.6959(13)
Bi(1)-N(3)	2.518(5)	2.4499(13)
Bi(1)-N(4)	2.455(5)	2.4171(13)
Bi(1)-O(1)	2.796(4)	2.4334(11)
Bi(1)-O(3)	2.561(4)	2.7549(12)

Bi(1)-O(5)	2.243(4)	2.3071(11)
Bi(1)-O(7)	2.453(4)	2.5510(12)
Bi(1)-O(1w)	2.858(5)	

Radiochemistry

General

All solvents and reagents that were used for the radiochemical experiments possessed high purity to avoid metallic contamination. Ultrapure (u.p.) water and u.p. HNO_3 were purchased form Carl Roth. The Pb-foil were obtained from Alfa Aesar. The $^{205/206}$Bi radioisotope was prepared in GE PETtrace cyclotron at the Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, University of Debrecen, Hungary. Activity measurements were carried out with a CAPINTEC CRC-15PET dose calibrator and a Perkin Elmer Packard Cobra gamma counter. Radio-TLC was performed on iTLC plate (form Waters) and analyzed using MiniGita TLC-Scanner with GINA-Star TLC soft-ware. TK-200 resin was purchased from Triskem. The purification of the labelled compounds was carried out with a Waters SepPak® C18 plus Light cartridge.

Preparation and purification of $^{205/206}$Bi isotopes

The $^{205/206}$Bi isotope mixture was produced in a GE PETtrace cyclotron with 16 MeV beam on natural Pb-foil target (99.995%, 0.9 by 0.9 cm, 0.25 mm thick). A 1 hour irradiation with a 20 μA beam current yielded approximately 80 MBq activity. After a 24 h decay period, the irradiated Pb target was dissolved in 2 mL of 7 M HNO_3. The solution was concentrated to ~500 μL, while $Pb(NO_3)_2$ was precipitated. The solution was separated from the solid and diluted to 5 mL with water and filtered with Millipore 0.22-μm filter. This solution was transferred onto a column self-filled with TK 200 resin (70 mg). The TK 200 column was preconditioned with 0.7 M u.p. HNO_3 (1 mL), 7 M u.p. HNO_3 (3 mL), and 0.7 M u.p. HNO_3 (5 mL). After the loading of the $^{205/206}$Bi isotope containing solution, the column was washed with 5 mL of 0.7 M u.p. HNO_3 to remove the remaining Pb target materials, and the $^{205/206}$Bi isotopes were eluted with 7 M u.p. HNO_3 in 1 mL fractions. The fractions which contained $^{205/206}$Bi isotopes (~40 MBq) were concentrated to dryness and were redissolved in 300-400 μL of 0.1 M u.p. HCl and used for radiolabeling experiments.

Investigation of $^{205/206}$Bi labeling efficiency of chelators using different ligand concentrations (1, 5, 10, 100, 300 and 1000 μM) at 37 and 95 °C

A volume of 35 μL of 3 M NH_4OAc buffer (pH 4) and 5 μL of OCTAPA or CHX-OCTAPA or CPX-OCTAPA aqueous solution with different concentrations were added to 10 μL $^{205/206}$Bi in 0.1 M HCl (~1 MBq). The applied ligand concentrations in the mixtures were as follows: 0.001, 0.002, 0.005, 0.01, 0.1 and 1 mM for each chelator. The reaction mixtures were heated at 37 and 95 °C, respectively. Then the radiochemical purity of the mixtures were analyzed by instant thin-layer chromatography using iTLC plate developed with a 0.5 M citrate solution (pH 5.5). Each experiment was performed in triplicate.

Radiolabeling of H_4OCTAPA, H_4*Cp*OCTAPA and H_4*CHX*OCTAPA with $^{205/206}$Bi

Radiolabeling for all ligands was performed as follows: 350 μL of NH_4OAc buffer (3 M, pH 4) and 50 μL of a 1 mM aqueous solution of the ligand were added to 100 μL of $^{205/206}$Bi in 0.1 M HCl (~15 MBq) solution. The reaction mixture was heated at 37 °C for 15 minutes and then was passed through a SPE cartridge (SepPak® C18 Plus Light, Waters) preconditioned with 5 mL of ethanol and 5 mL of water. After purging of the cartridge with 1 mL of water, the labeled complex was eluted with 200 μl of 96 v/v% ethanol. This solution was concentrated to dryness and the [$^{205/206}$Bi(*CHX*OCTAPA)] was redissolved in 400 μL of water and used for stability studies. The radiochemical purity of the [$^{205/206}$Bi(*CHX*OCTAPA)] was determined by instant thin-layer chromatography using the above-mentioned radio-TLC method.

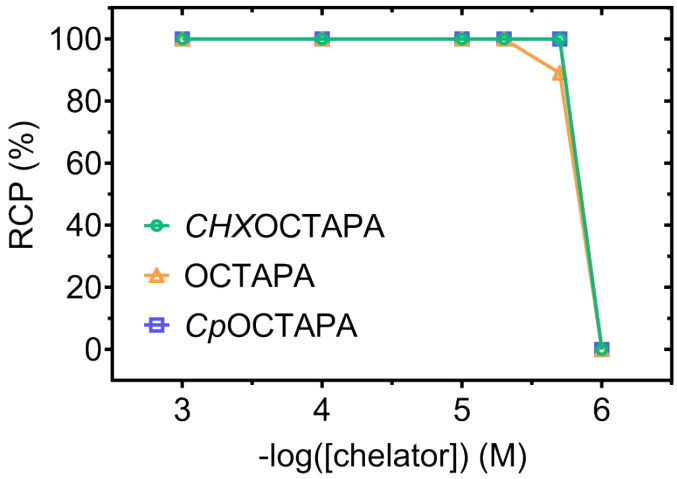


Figure S27: Labeling efficiency of the ligands at 95 °C

Stability test of [$^{205/206}$Bi(OCTAPA)], [$^{205/206}$Bi(*CHX*OCTAPA)] and [$^{205/206}$Bi(*Cp*OCTAPA)] in rat serum

100 µL of aqueous solution of each $^{205/206}$Bi labeled complex was added to 100 µL rat serum, respectively. The mixtures were analyzed at the beginning and after 0.5, 1, 2, 3 and 24 hours by radio-TLC method as described above. Each experiment was performed in triplicate.

DTPA challenge of [$^{205/206}$Bi(OCTAPA)], [$^{205/206}$Bi(*CHX*OCTAPA)] and [$^{205/206}$Bi(*Cp*OCTAPA)]

50 µL of aqueous solution of each ^{radio}labeled complex was incubated with 50 µL of 12 mM DTPA (pH 7.4) solution at room temperature. The mixtures were analyzed by radio-TLC method as mentioned above at the beginning and after 0.5, 1, 2, 3 and 24 hours incubation time. Each experiment was performed in triplicate.

Metal challenge of [$^{205/206}$Bi(OCTAPA)], [$^{205/206}$Bi(*CHX*OCTAPA)] and [$^{205/206}$Bi(*Cp*OCTAPA)]

49 µL of aqueous solution of each $^{205/206}$Bi labeled complex was incubated with 1 µL of the 1:1 mixture of 0.1 mM $ZnCl_2$ and 0.01 mM $CuCl_2$ and 50 µL of the 1:1 mixture of 1.02 mM $MgCl_2$ and 2.28 mM $CaCl_2$ at room temperature. Samples were taken from the mixtures at the beginning and after 0.5, 1, 2, 3 and 24 hours. For the analysis of the samples the above described radio-TLC method was applied. Each experiment was performed in triplicate.

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