Supporting Information

Triple Threat Bismuth Peptide Imaging in Cells

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Instrumentation and materials used for fluorophore synthesis

All chemicals and reagents were purchased from reputable commercial suppliers and used without further purification. All reactions were performed under a nitrogen atmosphere unless stated otherwise. Reactions were heated using either a silicone oil bath or an aluminium heating block placed on a magnetic stirring hotplate, equipped with an electronic contact thermometer to maintain the specified temperatures. Reactions were monitored by thin-layer chromatography on silica gel pre-coated aluminium plates (Merck, TLC Silica gel 60 F_{254}). TLC chromatograms were visualised by UV illumination or developed with potassium permanganate stain. All column chromatography was performed on pre-packed flash cartridges (iLOK, silica gel 40-63 μm, 60 Å) using a Biotage Isolera One or a Biotage Selekt.

All NMR spectra were recorded at 300 K on a Bruker NEO 300 or a Bruker AVANCE NEO 500. Deuterated solvents were purchased from Sigma Aldrich. Chemical shifts were reported in parts per million (ppm) and coupling constants were reported in Hertz (Hz). ^{1}H and ^{13}C chemical shifts (δ) were referenced to solvent residual signals. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = doublet of doublets, m = multiplet, br = broad), coupling constant(s) (*J*) and relative integral. All ^{13}C NMR spectra were proton decoupled.

Low resolution electrospray ionisation (ESI) mass spectrometry was performed on a Bruker AmaZon SL Ion Trap Mass Spectrometer. High resolution ESI mass spectrometry was obtained at the Sydney Mass Spectrometry Facility at the University of Sydney using a Bruker solariX 2xR Fourier Transform Ion Cyclotron Resonance Mass Spectrometer.

UPLC-MS was performed on a Shimadzu UPLC-MS instrument with an LC-M20A pump, SPD-M30A diode array detector, and a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode.

Analytical reversed-phase UPLC was performed on a Waters ACQUITY™ Premier UPLC system equipped with a PDA eλ Detector (λ = 210 – 400 nm). Separations were performed on a Waters ACQUITY™ Premier BEH C8 VanGuard™ FIT Column, 1.7 μm, 2.1 mm × 50 mm at 40 ºC at a flow rate of 0.5 mL/min. All separations were performed using a mobile phase of 0.1 vol.% formic acid in H_2O (Solvent A) and 0.1 vol.% formic acid in MeCN (Solvent B) using linear gradients.

The rhodamine B fluorophore was synthesised and attached to peptides as previously described.[1] 7-(Diethylamino)coumarin-3-carboxylic acid is commercially available. All other fluorophores were synthesised as described below.

Instrumentation and materials used for peptide synthesis

All high-resolution mass spectrometry (HRMS) experiments were conducted on a Thermo-Fischer Scientific Orbitrap Elite Hybrid Ion Trap-Orbitrap equipped with an UltiMate 3000 HPLC. The LC chromatograms were recorded on an Agilent 1290 Infinity equipped with a reverse-phase column ZORBAX Eclipse XDB-C18 Rapid resolution (2.1 × 5.0 mm, 1.8 μm) (925700-932) in conjunction with a guard column Poroshell 120 EC-C18 (2.1 × 5 mm 2.7 μm) (column I) and were held at 25 °C. Elution was monitored by UV absorbance (214 nm and 254 nm) and MS module. Chromatograms were plotted using GraphPad Prism 10. Peptides were purified by preparative HPLC using either an Agilent 1260 Infinity Binary LC equipped with a Macherey Nagel VP 250/32 NUCLEODUR C18 HTEC, 5 μm (column II) at a flowrate of 30 mL/min or Waters Alliance Separation Module 2690, with a Waters 996 photodiode array detector equipped with a Grace Alltima, C18, 5 μm, 22 × 250 mm (column III) at a flowrate of 15 mL/min. Elution was monitored in both cases by UV absorbance (210 nm, 254 nm, 405 nm, 454 nm and 561 nm). Solid-phase peptide synthesis was conducted using Rink amide resin (Rink Amide MBHA resin LL, 100-200 mesh) with a capacity of 0.41 mmol/g, Merck, Novabiochem (Germany) and Fmoc-protected amino acids (GL Biochem, China) and used without further purification. All other reagents were purchased either from Sigma Aldrich (Germany) or AK Scientific (CA, United States).

Analytical LC-MS method:

This method employed a gradient system of MeCN/H_2O (0.1% formic acid) at a flow rate of 0.3 mL/min and used column I. The gradient started isocratic at 5% MeCN for 2 min and increased gradually to 95% MeCN over 10 min. The final solvent composition was retained for 3 min until the end of the run.

Preparative HPLC methods

Method A. This method was employed for the purification of **Bi-CPP-1** and **Bi-CPP-3** using column II and a gradient system of MeCN/H_2O (0.1% formic acid). The gradient started isocratic at 1% MeCN for 10 min, before it was increased to 95% MeOH over 2 min.

Method B. This method was employed for the purification of **Bi-CPP-1-Np** and **Bi-CPP-1-Cou** using column III and a gradient system of MeOH/H_2O (0.1% formic acid). The gradient started isocratic for 2 min at 5% MeOH and was increased to 13% over 13 min and then to

28% over 30 min. Subsequently, the gradient was increased to 38% MeOH over 10 min and then to 99% over 2 min.

Method C. This method was employed for the purification of **Bi-CPP-1-NpBr** and **Bi-CPP-1-CouBr** using column III and a gradient system of MeOH/H_2O (0.1% formic acid). The gradient started isocratic for 2 min at 5% MeOH and was increased to 13% over 13 min and then to 38% over 40 min. Subsequently, the gradient was increased to 48% MeOH over 10 min and then to 99% over 2 min.

Method D. This method was employed for the purification of **Bi-CPP-1-RhB**, **Bi-CPP-1-RhBBr**, **Bi-CPP-3-RhB** and **Bi-CPP-3-RhBBr** using column III and a gradient system of MeOH/H_2O (0.1% formic acid). The gradient started at 5% MeOH and was increased to 15% over 15 min and then to 20% over 5 min. The MeOH content was then gradually increased to 40% over 30 min. Finally, the gradient was increased to 50% within 10 min and then to 99% over 2 min.

Method E. This method was employed for the purification of **Bi-CPP-2** using column III and a gradient system of MeOH/H_2O (0.1% formic acid). The gradient started isocratic for 5 min at 5% MeOH and was increased to 10% over 5 min and held at 10% for 5 min. Subsequently the gradient was increased to 25% over 30 min and then to 35% over 10 min. Finally, the gradient was increased to 99% over 2 min.

Method F. This method was employed for the purification of **Bi-CPP-2-Np** using column III and a gradient system of MeOH/H_2O (0.1% formic acid). The gradient started at 5% MeOH and was increased to 11% over 3 min and then to 21% over 10 min. Subsequently, the gradient was increased to 41% MeOH over 40 min and then to 51% over 10 min before increased up to 99% over 2 min.

Method G. This method was employed for the purification of **Bi-CPP-2-NpBr** using column III and a gradient system of MeOH/H_2O (0.1% formic acid). The gradient started isocratic for 2 min at 5% MeOH and was increased to 15% over 5 min and then to 25% over 10 min. Subsequently, the gradient was increased to 45% MeOH over 40 min and then to 55% over 10 min before increased up to 99% over 2 min.

General procedure for solid-phase peptide synthesis

In a 10 mL polypropylene syringe equipped with a filter, Rink amide resin was swollen in CH_2Cl_2 for 30 min. The resin was then washed with DMF (×3) before Fmoc deprotection with 20% piperidine in DMF for 10 min (×2). The resin was then washed with DMF (×3), CH_2Cl_2 (×3), and DMF (×3). The resin was treated with a solution of *N*-Fmoc-protected amino acid (3

eq.), HBTU (3 eq.), HOBt (3 eq.), and DIPEA (4 eq.) in DMF (1.5 mL per 125 mg Rink amide resin). The reaction mixture was shaken before being washed with DMF (×3), CH_2Cl_2 (×3), and DMF (×3). The first ten amino acids were coupled for 1.5 h, while subsequent amino acids were coupled twice for 45 min. Fmoc deprotection, washing, and coupling steps were repeated until the desired peptide sequence was completed. An *N*-terminal fluorophore was installed by treatment with the fluorophore carboxylic acid (1.5 eq.), HBTU (1.5 eq.), HOBt (1.5 eq.) and DIPEA (2 eq.) for 1.5 h. The *N*-terminus of **Bi-CPP-1**, **Bi-CPP-2** and **Bi-CPP-3** was acetylated by treatment with pyridine (50 eq.) and acetic anhydride (50 eq.) in CH_2Cl_2 for 1 h. After installation of the *N*-terminal cap, the resin was washed with DMF (×3), CH_2Cl_2 (×3), Et_2O (×3), and dried under reduced pressure for 2 h. The peptide was cleaved from the resin using a mixture (1.5 mL per 125 mg Rink amide resin) of TFA (89%), TIPS (3%), EDT (3%), thioanisole (5%), and H_2O (3%). The mixture was shaken for 2 h. The cleavage solution was poured into ice-cold Et_2O (35 mL). The precipitate was centrifuged and washed with cold Et_2O (×2). The Et_2O was decanted, and the crude peptide was dried under reduced pressure overnight. The product was purified by preparative HPLC. The fractions containing the product were combined and lyophilised to yield the linear peptides. All purified peptides were stored at -20 °C. Identity was confirmed by HRMS.

Synthesis and isolation of cell penetrating bismuth-peptide bicycles

Purified linear peptides with fluorescent dyes attached were dissolved in H_2O/MeCN (50:50) at a concentration of 5.0 mM. A solution of TCEP-NaOH was added stepwise to the peptide to yield a final solution of 2.5 mM peptide, 15 mM TCEP-NaOH, pH 7.5 H_2O/MeCN (75:25). Purified peptides without dye were dissolved in H_2O at a concentration of 5.0 mM. A solution of TCEP-NaOH was added stepwise to the solution to yield 2.5 mM peptide, 15 mM TCEP-NaOH, pH 7.5 H_2O. 1.2 equivalents of $BiBr_3$ (0.5 M DMSO stock) were added to the reduced linear peptides. The mixtures were centrifuged at 2000 × *g* for 3 min and the supernatant containing bicyclic product was purified using anion exchange resin.

Preparation of anion exchange column and purification of the bicyclic product

In a polypropylene syringe equipped with a filter, Sephadex A-25 (Cytiva) was swollen in H_2O and allowed to swell under gentle shaking for at least 3 h. The swollen resin was extensively washed with H_2O until the flowthrough reached pH 7. Subsequently, the column was washed with 20 column volumes (using either Ultra-pure H_2O or Ultra-pure H_2O/MeCN (75:25)) and shaken for 30 min. Finally, the resin was washed with 20 column volumes of either Ultra-pure

H_2O or Ultra-pure H_2O/MeCN (75:25). A clean filter was placed on top of the resin to prevent the column from drying out and enabling facile application of the peptide mixture. The peptide mixture was loaded onto the column and eluted using either Ultra-pure H_2O or Ultra-pure H_2O/MeCN (75:25), depending on the peptide's hydrophobicity/solubility. The flowthrough was collected and fractions containing the bicyclic products were merged, filtered through a 0.2 μm filter and lyophilised. Identity was confirmed by high-resolution mass spectrometry.

Cell culture

Human ovarian cancer cells (SKOV-3) were cultured using Advanced Dulbecco's Modified Eagle's Medium (ADMEM, Thermo Fisher Scientific) with 2% foetal bovine serum (FBS, Thermo Fisher Scientific) and 2.5 mM L-glutamine (Thermo Fisher Scientific). Cells were incubated at 37 ºC in an atmosphere containing 5% carbon dioxide. ~5000 cells were seeded onto sterilised silicon nitride windows (Silson, 500 nm thick, 2 × 2 mm), then allowed to adhere for 12 h before media was removed and replaced with media containing 10 μM of bismuth-peptide (Bi peptide). Stock solutions of Bi peptides (1 mM) were prepared in ultrapure water (MilliQ) immediately prior to diluting in treatment media. Following 4 h of treatment, cells were chemically fixed according to protocols previously described.[2]

MTT assays

SKOV-3 cells were seeded into 96-well plates (flat bottom, clear, Costar) at a density of 2,500 cells/well in 200 μL of supplemented ADMEM. The cells were allowed to adhere for 48 h. Stock solutions of Bi-CPP peptides were freshly prepared in water. A serial dilution was performed to prepare dosing solutions of the peptides at final concentrations of 128, 64, 32, 16, 8, 4, 2, and 1 μM in supplemented ADMEM. The cell culture media was aspirated from the wells and replaced with 100 μL of either fresh supplemented ADMEM (control) or ADMEM containing peptide. Following the 24 h incubation, 20 μL of MTT reagent (2.5 mg/mL in PBS pH 7.4, Sigma Aldrich) was added to each well. The plates were incubated for a further 4 h. The solution was then carefully removed, followed by the addition of 150 μL of DMSO. The plates were shaken at 300 rpm for 60 s before the absorbance at 600 nm were measured in a Revvity Envision 2105 plate reader. Cell viability values were normalised to the untreated control (containing ADMEM only). The data is presented as the average percentage viability of four individual technical replicates from a single experiment.

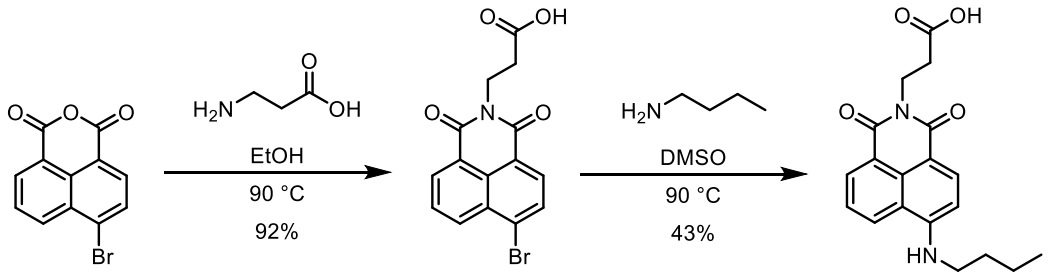
Optical confocal microscopy

Brightfield and optical fluorescence images of the cells fixed on windows were collected on a Zeiss LSM980 super-resolution confocal microscope with Airyscan 2 (Germany) using a 20× objective (~0.124 μm pixel size). For Bi peptides containing naphthalimide (Np), coumarin (Cou) or rhodamine B (RhB) appendages, fluorescence was obtained with excitation of 488 nm, 354 nm, and 558 nm (respectively) and emission ranges of 550 – 550 nm, 446 – 579 nm, and 491 – 676 nm (respectively). These images were obtained prior to X-ray fluorescence imaging.

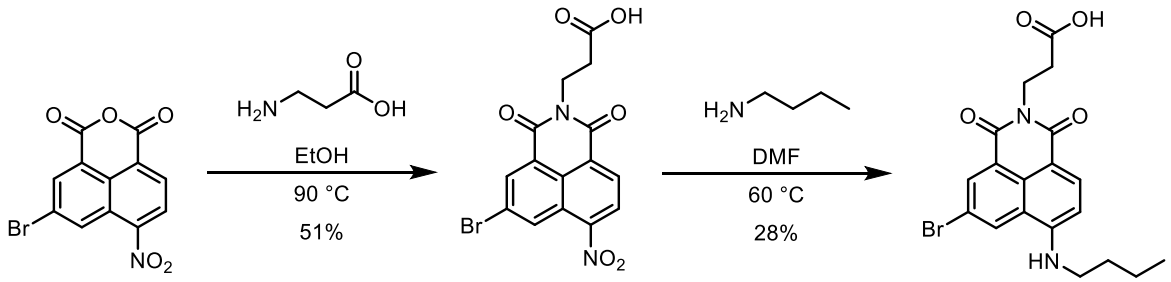
X-ray fluorescence microscopy

X-ray fluorescence imaging was conducted at the Australian Synchrotron X-ray Fluorescence (XFM) beamline, Victoria, Australia.[3] The incident X-ray energy of the beamline was tuned to 15.8 keV using a Si(111) monochromator and the beam spot was focussed to ~1 μm using a Kirkparick-Baez mirror pair. The fluorescence signal was collected using a Vortex ME3 detector (Hitachi High-Tech, USA) with a 90º collection angle geometry relative to the incident beam direction. The data was collected over two trips to the Australian Synchrotron with different data acquisition parameters; control measurements were collected for both parameter sets. The fluorescence signal of Bi peptides containing Np was collected with a dwell of 100 ms at a 1-μm step size. For the Cou and RhB derivatives, fluorescence was collected with a dwell of 8.6 ms and a 0.5-μm step size. GeoPIXE (v7.7) and Fiji ImageJ (v 1.54f) software[4] was used to analyse the elemental maps and perform quantitative analysis. Quantification was determined from the mean areal density (ng/cm^2) value per cell, with regions of interest (ROIs) for each cell determined by locating the same cells on the brightfield optical images obtained from confocal microscopy. Ordinary one-way ANOVA and Tukey's multiple comparison test with a single pooled variance was used. Statistical analysis by ANOVA was performed using GraphPad Prism (version 8). False colour images for fluorophores, Bi, Br and Zn were generated with Fiji ImageJ and Python 3, by applying the "Fire" colour scheme and a Gaussian blur filter of $\sigma = 1$ for fluorophores, $\sigma = 0.5$ for Bi and Br, and $\sigma = 1$ for Zn.

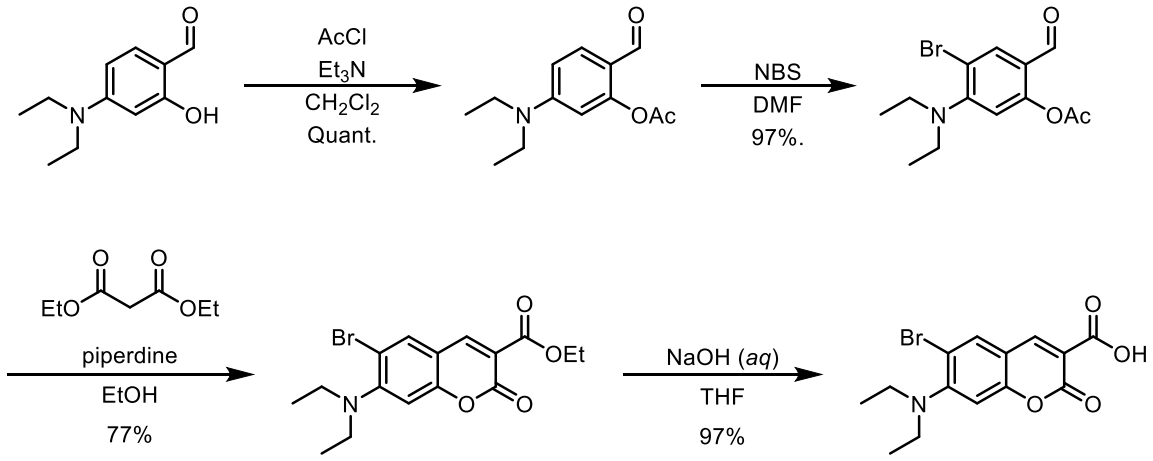
Synthesis and analytical data of fluorescent dyes



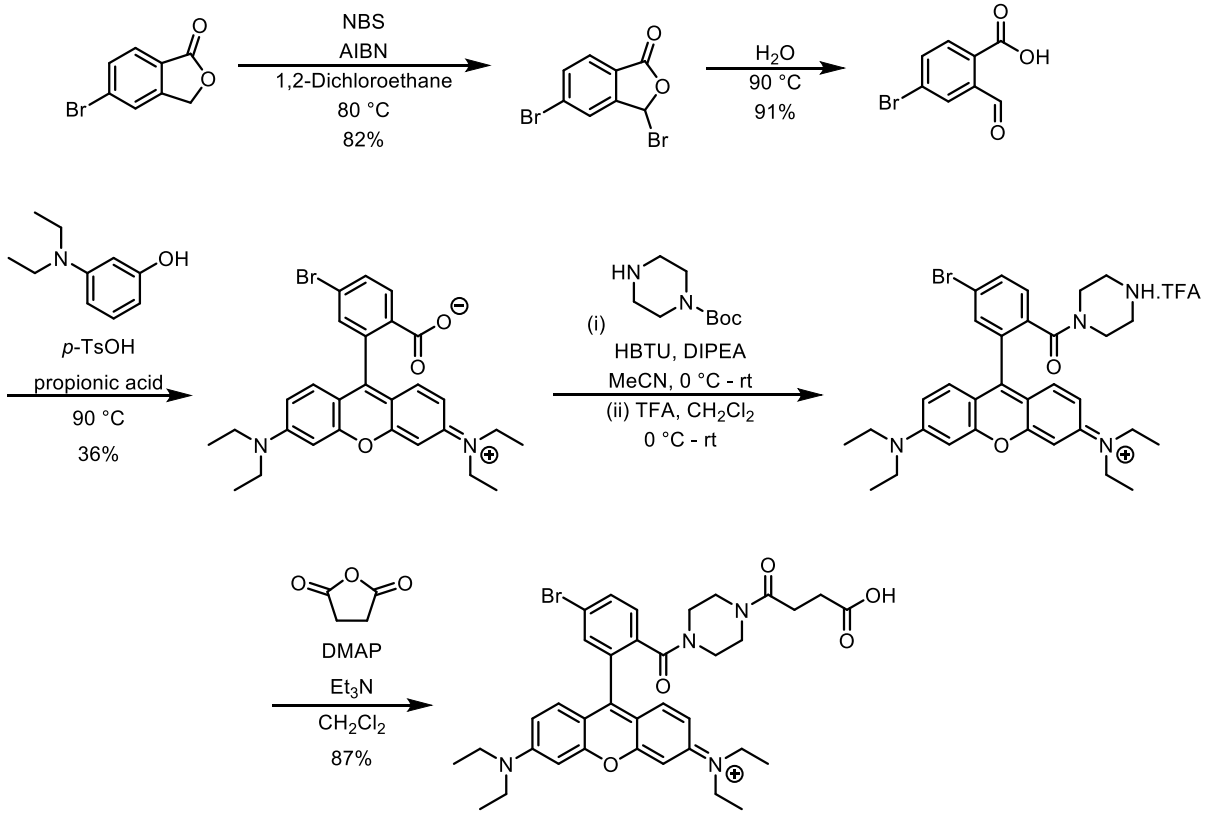
Scheme 1. Synthesis of naphthalimide dye (**Np**).



Scheme 2. Synthesis of Br-naphthalimide dye (**NpBr**).

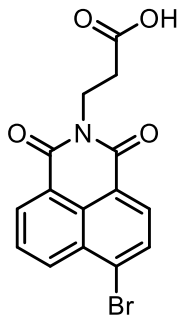


Scheme 3. Synthesis of Br-coumarin dye (**CouBr**).



Scheme 4. Synthesis of Br-rhodamine dye (**RhBr**).

***N*-Propanoate-4-bromo-1,8-naphthalimide:**

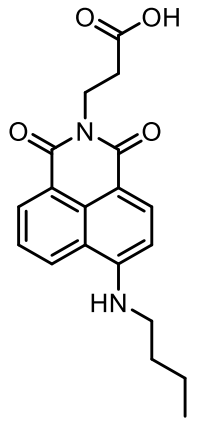


4-Bromo-1,8-naphthalic anhydride (2.77 g, 10.0 mmol) was suspended in absolute EtOH (100 mL). *β*-Alanine (899 mg, 10.1 mmol) was added and the resultant mixture heated to reflux (90 °C) and stirred for 16 h. The reaction mixture was cooled to rt and the resultant precipitate isolated by vacuum filtration and washed with EtOH. Purification by recrystallisation from EtOH gave the desired product as a beige solid (3.22 g, 92%).

^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 12.29 (s, 1H), 8.50 (d, *J* = 7.3 Hz, 1H), 8.47 (d, *J* = 8.6 Hz, 1H), 8.26 (d, *J* = 7.9 Hz, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 7.94 (dd, *J* = 8.5, 7.3 Hz, 1H), 4.23 (t, *J* = 7.7 Hz, 2H), 2.59 (t, *J* = 7.7 Hz, 2H).
^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 172.4, 162.7, 162.7, 132.6, 131.5, 131.3, 130.9, 129.7, 129.1, 128.7, 128.2, 122.7, 121.9, 35.9, 32.1.

Data in accordance with literature values.[5]

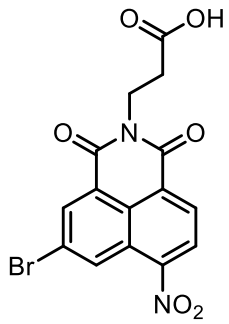
***N*-Propanoate-4-butylamino-1,8-naphthalimide:**



N-Propanoate-4-bromo-1,8-naphthalimide (870 mg, 2.50 mmol) was dissolved in DMSO (20 mL). The reaction vessel was then placed under high vacuum for 10 min before being backfilled with a nitrogen atmosphere. ^{n}BuNH$_2$ (988 µL, 10.0 mmol) was added dropwise and the resultant solution heated to 90 °C and stirred for 16 h. The reaction mixture was then diluted with EtOAc (100 mL) and washed with 1M HCl (*aq*) (100 mL). The aqueous phase was back extracted with EtOAc (2 × 100 mL) and the combined organics washed with 1M HCl (*aq*) (100 mL) and brine (250 mL). The organic phase was then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification *via* flash chromatography (silica, 1 – 15% MeOH in CH_2Cl_2) gave the desired product as a yellow solid (363 mg, 43%).

^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 12.27 (br. s, 1H), 8.67 (dd, *J* = 8.6, 1.2 Hz, 1H), 8.39 (d, *J* = 7.3, 1.0 Hz, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 7.71 (t, *J* = 5.5 Hz, 1H), 7.63 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.72 (d, *J* = 8.7 Hz, 1H), 4.22 (t, *J* = 7.7 Hz, 2H), 3.35 (td, *J* = 7.1, 5.3 Hz, 2H), 2.54 (t, *J* = 7.7 Hz, 2H), 1.75 - 1.62 (m, 2H), 1.50 - 1.35 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H).
^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 172.6, 163.7, 162.8, 150.8, 134.3, 130.6, 129.5, 128.7, 124.2, 121.7, 120.1, 107.3, 103.7, 42.6, 35.4, 32.5, 30.0, 19.9, 13.8.
HRMS (ESI): calculated for $C_{19}H_{20}N_2O_4Na$ $[M+Na]^+$ 363.1315, found 363.1314.

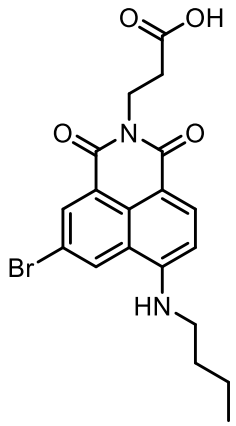
***N*-Propanoate-3-bromo-5-nitro-1,8-naphthalimide:**



3-Bromo-5-nitro-1,8-naphthalic anhydride (644 mg, 2.00 mmol) was suspended in absolute EtOH (20 mL). β-Alanine (180 mg, 2.02 mmol) was added and the resultant mixture heated to reflux (90 °C) and stirred for 16 h. The reaction mixture was cooled to rt and the resultant precipitate collected by vacuum filtration and washed with EtOH. Purification by recrystallisation from EtOH gave the desired product as an orange/beige solid (400 mg, 51%).

^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 12.41 (s, 1H), 8.92 (d, *J* = 1.8 Hz, 1H), 8.61 (s, 2H), 8.59 (d, *J* = 1.9 Hz, 1H), 4.29 – 4.20 (m, 2H), 2.61 (t, *J* = 7.7 Hz, 2H).
^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 172.3, 161.8, 161.7, 147.7, 133.6, 130.9, 129.8, 127.1, 127.0, 125.9, 124.7, 124.0, 123.5, 36.1, 31.8.

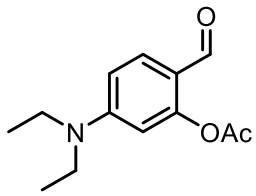
***N*-Propanoate-3-bromo-5-butylamino-1,8-naphthalimide:**



N-Propanoate-3-bromo-5-nitro-1,8-naphthalimide (200 mg, 0.51 mmol) was dissolved in anhydrous DMF (5 mL). The resultant solution was degassed under high vacuum for 10 min then placed under a nitrogen atmosphere. $^{n}BuNH_2$ (202 µL, 2.04 mmol) was added dropwise and the resultant solution heated to 60 °C and stirred for 16 h. The reaction mixture was then diluted with EtOAc (75 mL), washed with 1M HCl (*aq*) (3 × 50 mL), and brine (50 mL). The organic phase was then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification *via* flash chromatography (silica, 1 – 15% MeOH in CH_2Cl_2) gave the desired product as an orange solid (60 mg, 28%).

^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 12.30 (s, 1H), 8.96 (d, *J* = 2.0 Hz, 1H), 8.34 (d, *J* = 1.8 Hz, 1H), 8.21 (d, *J* = 8.6 Hz, 1H), 7.79 (t, *J* = 5.3 Hz, 1H), 6.76 (d, *J* = 8.7 Hz, 1H), 4.19 (t, *J* = 7.7 Hz, 2H), 3.35 (dd, *J* = 7.3, 5.5 Hz, 2H), 2.60 – 2.51 (m, 2H), 1.75 - 1.63 (m, 2H), 1.50 - 1.36 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H).
^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 172.5, 162.5, 162.3, 149.9, 134.6, 132.4, 130.7, 128.1, 123.7, 121.6, 117.1, 107.3, 104.6, 42.7, 35.5, 32.3, 29.8, 19.8, 13.8.
HRMS (ESI): calculated for $C_{19}H_{19}BrN_2O_4Na$ $[M+Na]^+$ 441.0420, found 441.0421.

2-(Acetyloxy)4-(diethylamino)benzaldehyde:

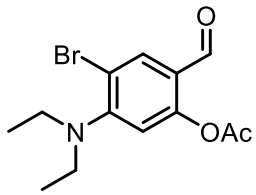


4-(Diethylamino)salicylaldehyde (2.00 g, 10.4 mmol) was dissolved in anhydrous CH_2Cl_2 (40 mL), under a nitrogen atmosphere. Et_3N (3.03 mL, 21.7 mmol) was added, and the resultant solution cooled to 0 °C. AcCl (1.10 mL, 15.5 mmol) was added dropwise, and the reaction mixture stirred for 1 h. The ice bath was then removed, and the reaction mixture allowed to warm to rt, and stirred for 4 h. The reaction mixture was then diluted with EtOAc (150 mL), washed with H_2O (2 × 50 mL), brine (50 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give the desired product as a dark orange/red oil (2.45 g, Quant.).

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 6.56 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.28 (d, *J* = 2.3 Hz, 1H), 3.42 (q, *J* = 7.1 Hz, 4H), 2.38 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 6H).

Data is in accordance with literature values.[6]

2-(Acetyloxy)-5-bromo-4-(diethylamino)benzaldehyde:

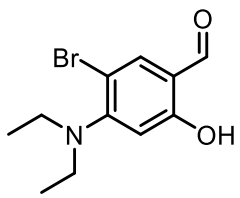


2-(Acetyloxy)4-(diethylamino)benzaldehyde (2.45 g, 10.4 mmol) was dissolved in anhydrous DMF (25 mL) under a nitrogen atmosphere. NBS (2.03 g, 11.4 mmol) in DMF (15 mL) was then added dropwise, and the reaction mixture stirred for 16 h (TLC analysis showed complete conversion of starting material). The reaction mixture was then diluted with EtOAc (100 mL), washed with brine (5 × 100 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo,* to give the desired product as a brown oil (3.16 g, 97%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.87 (s, 1H), 8.01 (s, 1H), 6.71 (br. S, 1H), 3.30 (q, *J* = 6.9 Hz, 4H), 2.39 (s, 3H), 1.15 (t, *J* = 6.9 Hz, 6H).

Data is in accordance with literature values.[6]

5-Bromo-4-(diethylamino)-2-hydroxybenzaldehyde:

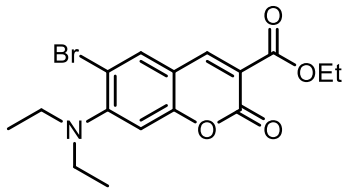


2-(Acetyloxy)-5-bromo-4-(diethylamino)benzaldehyde (3.16 g, 10.1 mmol) was dissolved in MeOH (50 mL), under a nitrogen atmosphere. K_2CO_3 (4.19 g, 30.3 mmol) was added, and the resultant mixture stirred for 4 h. The reaction mixture was then neutralised with 1M HCl (*aq*), diluted with H_2O (100 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The combined organics were then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification *via* flash chromatography (silica, 5 – 10% EtOAc in hexanes) gave the desired product as a yellow oil (1.50 g, 55%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 11.15 (s, 1H), 9.64 (s, 1H), 7.66 (s, 1H), 6.52 (s, 1H), 3.32 (q, *J* = 7.0 Hz, 4H), 1.15 (t, *J* = 7.0 Hz, 6H).

Data is in accordance with literature values.[6]

Ethyl 6-bromo-7-(diethylamino)-coumarin-3-carboxylate:

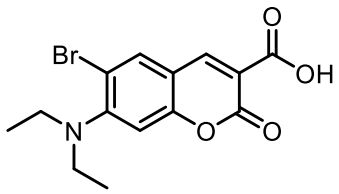


5-Bromo-4-(diethylamino)-2-hydroxybenzaldehyde (600 mg, 2.20 mmol) was dissolved in EtOH (15 mL), under a nitrogen atmosphere. Piperidine (20 μL, 0.22 mmol), then diethyl malonate (501 μL, 3.3 mmol) were added, and the resultant solution heated to 90 °C and stirred for 16 h. The reaction mixture was then diluted with H_2O (30 mL), extracted with EtOAc (3 × 30 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification *via* flash chromatography (silica, 2 – 20% EtOAc in hexanes) gave the desired product as a yellow solid (625 mg, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H), 7.76 (s, 1H), 6.90 (s, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 3.32 (q, *J* = 7.1 Hz, 4H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 6H)
^{13}C NMR (101 MHz, $CDCl_3$): δ 163.26, 156.84, 155.55, 155.17, 147.48, 134.37, 114.79, 113.71, 113.00, 108.83, 61.76, 46.09, 14.25, 12.30.

Data in accordance with literature values.[7]

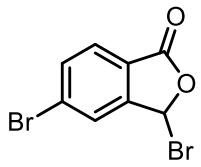
6-Bromo-7-(diethylamino)-coumarin-3-carboxylic acid:



6-Bromo-coumarin (58 mg, 0.16 mmol) was dissolved in THF (1 mL). 2 M NaOH (*aq*) (1 mL) was added dropwise, and the resultant mixture stirred for 16 h. TLC analysis (10% MeOH in CH_2Cl_2) indicated complete conversion. The reaction mixture was diluted with H_2O (10 mL), acidified with 5 M HCl (*aq*) (~1 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give the desired product as a yellow solid (53 mg, 97%).

^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 13.03 (br. s, 1H), 8.64 (s, 1H), 8.15 (s, 1H), 7.10 (s, 1H), 3.27 (q, J = 7.0 Hz, 4H), 1.05 (t, J = 7.0 Hz, 6H).
^{13}C NMR (126 MHz, $(CD_3)_2SO$): δ 164.0, 156.9, 155.0, 154.3, 147.7, 134.7, 114.8, 113.4, 113.2, 109.0, 45.6, 12.16.
HRMS (ESI): calculated for $C_{14}H_{14}BrNO_4Na$ $[M+Na]^+$ 361.9998, found 361.9998.

3,5-Dibromo-3H-isobenzofuran-1-one:

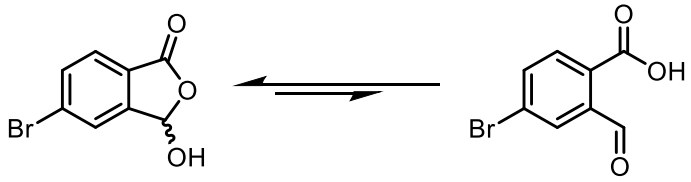


5-Bromophthalide (1.07 g, 5.00 mmol) was dissolved in 1,2-dichloroethane (25 mL) under a nitrogen atmosphere. NBS (979 mg, 5.50 mmol) was added portion-wise followed by AIBN (12% wt. in acetone) (~342 μL, 41 mg, 0.25 mmol). The resultant solution was heated to 80 °C and stirred for 16 h. The reaction mixture was concentrated and purification *via* flash chromatography (silica, 50 – 100% CH_2Cl_2 in hexanes) gave the desired product as a white solid (1.20 g, 82%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 – 7.72 (m, 3H), 7.34 (s, 1H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 166.4, 150.6, 134.7, 130.6, 127.3, 127.1, 123.1, 73.4.

Data in accordance with literature values.[8]

4-Bromo-2-formyl-benzoic acid:



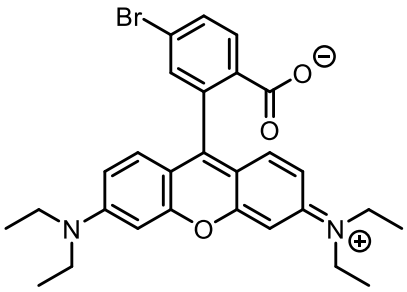
3,5-Dibromo-3H-isobenzofuran-1-one (1.10 g, 3.77 mmol) was suspended in H_2O (15 mL). The resultant suspension was heated to 100 °C and stirred for 4 h. The reaction mixture was then cooled to 4 °C for 16 h. The resultant precipitate was collected by vacuum filtration, washed with ice cold H_2O and hexanes to give the desired product as a white solid (782 mg, 91%).

^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 7.92 (d, J = 1.6 Hz, 1H), 7.84 (dd, J = 8.1, 1.6 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 6.65 (s, 1H).
^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 167.6, 149.4, 133.8, 128.5, 127.0, 126.4, 125.8, 97.7.

Note: In DMSO-d_6 the product is ~95% lactone.

Data in accordance with literature values.[8]

6-Br-Rhodamine B:

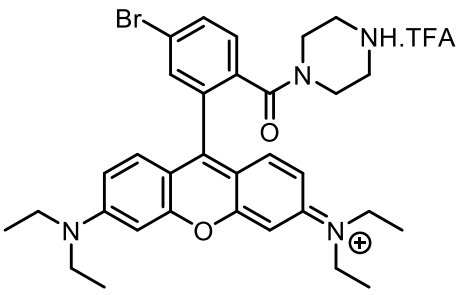


4-Bromo-2-formyl-benzoic acid (300 mg, 1.30 mmol), 3-diethylaminophenol (441 mg, 2.67 mmol), and *p*-TsOH (45 mg, 0.26 mmol) were dissolved in propionic acid (5.0 mL), under a nitrogen atmosphere. The resultant mixture was heated to 90 °C and stirred for 24 h. The reaction mixture was cooled to rt and then concentrated *in vacuo*. Purification *via* flash chromatography (silica, 1 – 15% MeOH in CH_2Cl_2) gave the desired product as a purple solid (246 mg, 36%).

^{1}H NMR (500 MHz, CD_3OD): δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.81 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.45 (d, *J* = 2.0 Hz, 1H), 7.24 (d, *J* = 9.5 Hz, 2H), 7.00 (dd, *J* = 9.5, 2.5 Hz, 2H), 6.90 (d, *J* = 2.6 Hz, 2H), 4.61 (s, 1H), 3.65 (q, *J* = 7.1 Hz, 8H), 1.29 (t, *J* = 7.1 Hz, 12H).
^{13}C NMR (126 MHz, CD_3OD): δ 172.2, 161.2, 159.4, 156.9, 140.4, 135.8, 133.8, 132.96, 132.94, 132.8, 124.6, 115.1, 114.8, 97.1, 46.7, 12.8

Data in accordance with literature values.[9]

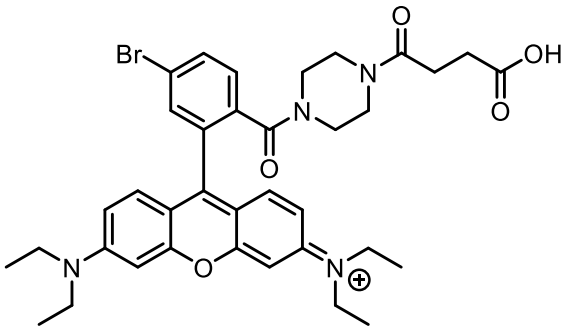
6-Br-Rhodamine B-piperazine:



6-Br-Rhodamine B (112 mg, 0.20 mmol) was dissolved in anhydrous MeCN (4 mL) and degassed by nitrogen sparging for 15 min. DIPEA (103 µL, 0.61 mmol) was added and the resultant solution cooled to 0 °C. HBTU (100 mg, 0.26 mmol) in MeCN (1 mL) was added slowly and the resultant solution stirred at 0 °C for 20 min. 1-Boc-piperazine (42 mg, 0.22 mmol) was added and the resultant mixture allowed to warm from 0 °C to ambient and stirred for 16 h. The solvent was removed *in vacuo* and the crude mixture dissolved in anhydrous CH_2Cl_2 (5 mL). TFA (2 mL) was added slowly, and the resultant mixture stirred for 16 h. The solvent was removed under a stream of nitrogen. Purification *via* flash chromatography (silica, 1 – 20% MeOH in CH_2Cl_2) gave the desired product as a purple solid (111 mg).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81 (d, *J* = 8.3 Hz, 1H), 7.48 (d, *J* = 5.9 Hz, 2H), 7.15 (d, *J* = 9.4 Hz, 2H), 6.95 (d, *J* = 9.6 Hz, 2H), 6.74 (s, 2H), 3.81 – 3.60 (m, 8H), 3.56 (dq, *J* = 15.1, 7.4 Hz, 4H), 3.09 (br. s, 2H), 3.03 (br. s, 2H), 1.31 (t, *J* = 7.3 Hz, 12H).
^{13}C NMR (126 MHz, $CDCl_3$): δ 166.4, 161.9 (q, *J* = 34.1 Hz), 157.8, 155.9, 153.5, 133.7, 133.4, 133.09, 133.06, 131.4, 129.4, 124.5, 117.1 (q, *J* = 294.6 Hz), 114.7, 113.5, 96.6, 46.3, 44.4, 43.1, 42.7, 38.7, 12.6.
HRMS (ESI): calculated for $C_{32}H_{38}BrN_4O_2$ $[M]^+$ 589.2173, found 589.2179.

6-Br-Rhodamine B-acid:



6-Br-Rhodamine B-piperazine (54 mg, 0.08 mmol) and DMAP (1.0 mg, 8.0 µmol) were dissolved in anhydrous CH_2Cl_2 (1.0 mL). Succinic anhydride (40 mg, 0.40 mmol) was added then Et_3N (45 µL, 0.32 mmol) was added dropwise. The resultant solution was stirred for 18 h then concentrated *in vacuo*. Purification *via* flash chromatography (silica, 5 – 50% MeOH in CH_2Cl_2) gave the desired product as a purple solid (48 mg, 87%).

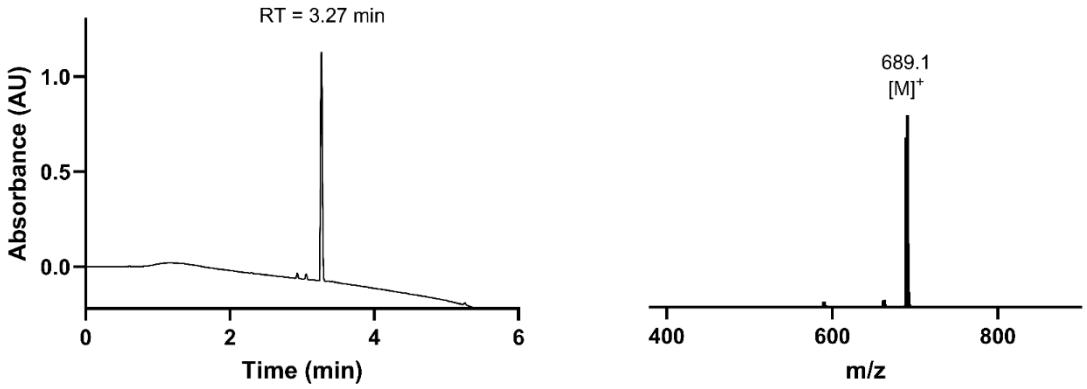
^{1}H NMR (500 MHz, CD_3OD): δ 7.95 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.29 (d, *J* = 9.5 Hz, 2H), 7.10 (d, *J* = 9.5 Hz, 2H), 6.97 (d, *J* = 2.5 Hz, 2H), 3.70 (q, *J* = 7.1 Hz, 8H), 3.54 – 3.33 (m, 8H), 2.56 (t, *J* = 7.0 Hz, 2H), 2.46 (t, *J* = 7.0 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 12H).

LCMS (ESI): calculated for $C_{36}H_{42}BrN_4O_5$ $[M]^+$ 689.2, found 689.1.

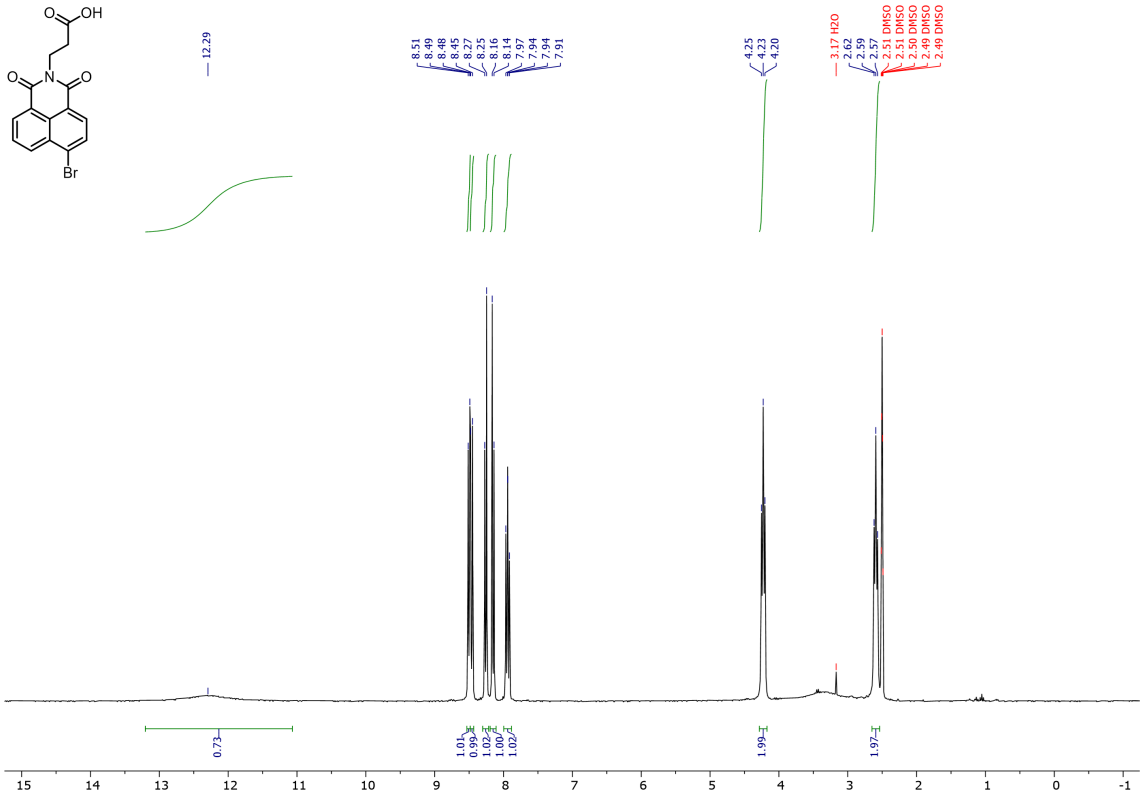
Analytical UPLC: t_R = 3.27 min, 96.7% purity (C_8, 1 – 100% MeCN in H_2O, with constant 0.1% v/v formic acid additive; 5 min run; flow rate = 0.5 mL/min; λ = 214 nm).

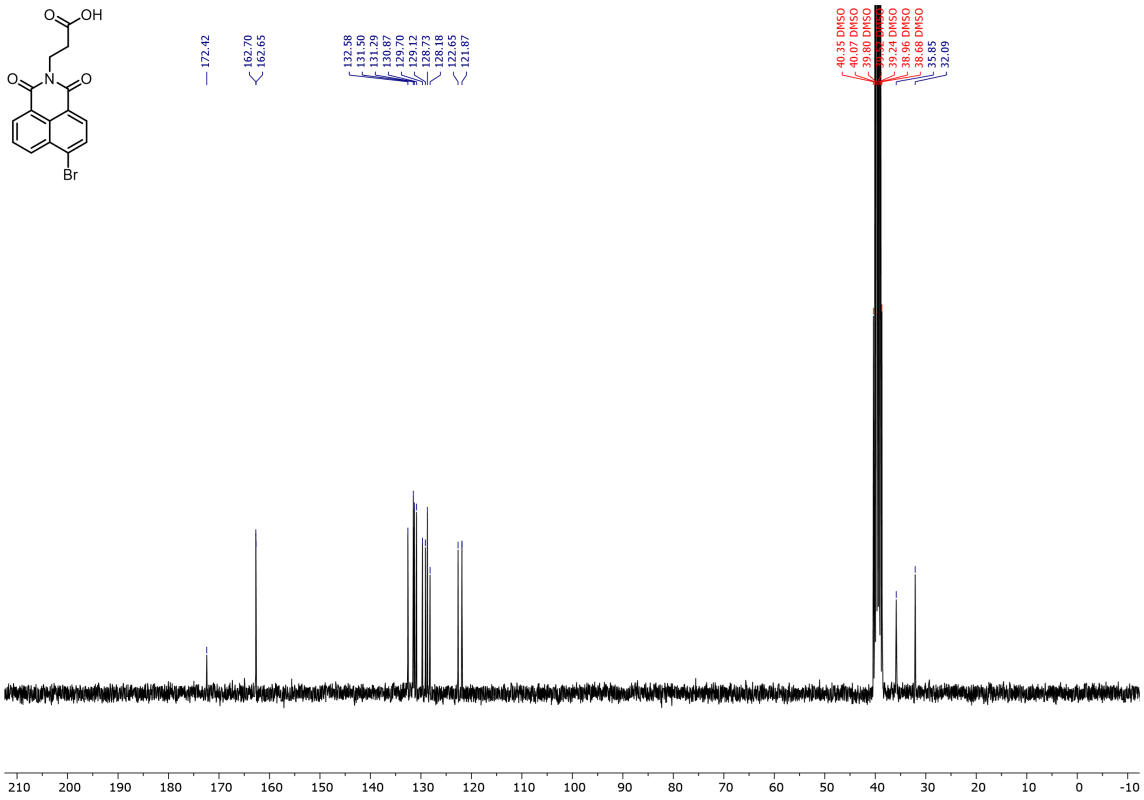
HRMS (ESI): calculated for $C_{36}H_{42}BrN_4O_5$ $[M]^+$ 689.2333, found 689.2335.

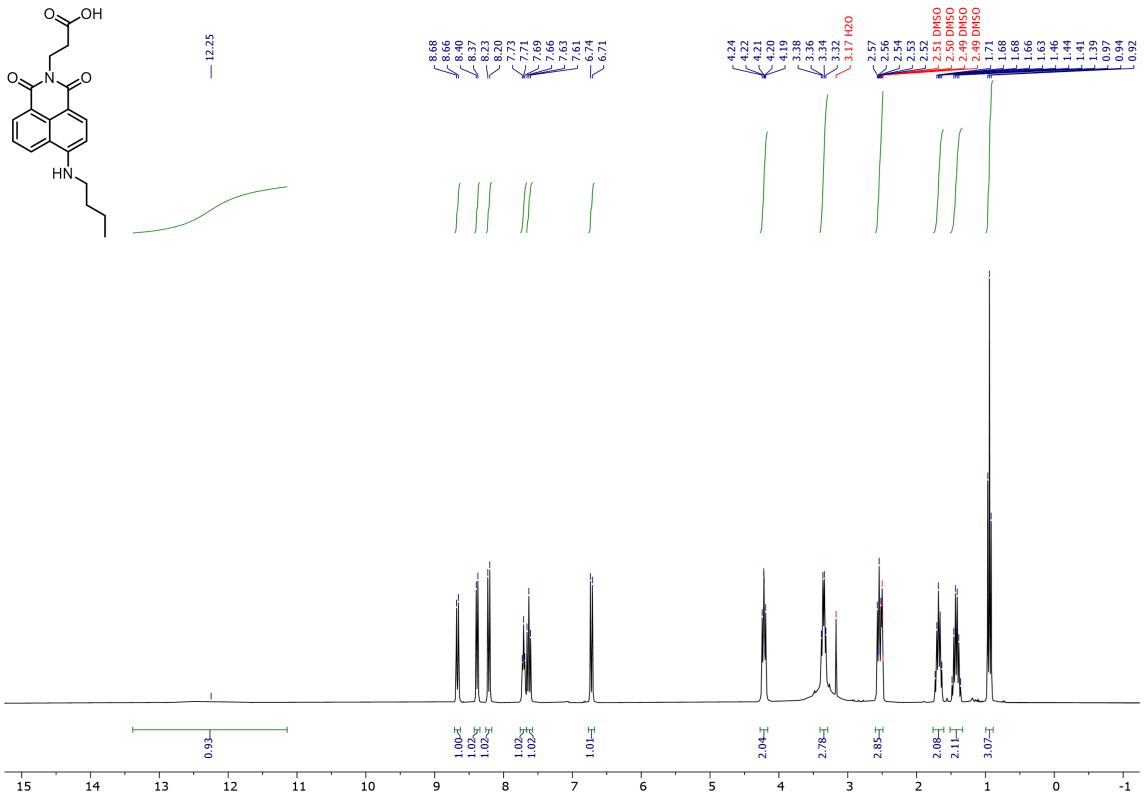
Note: The presence of rotamers prevented useful interpretation of the ^{13}C NMR spectra



NMR spectra of fluorescent dyes and synthetic precursors



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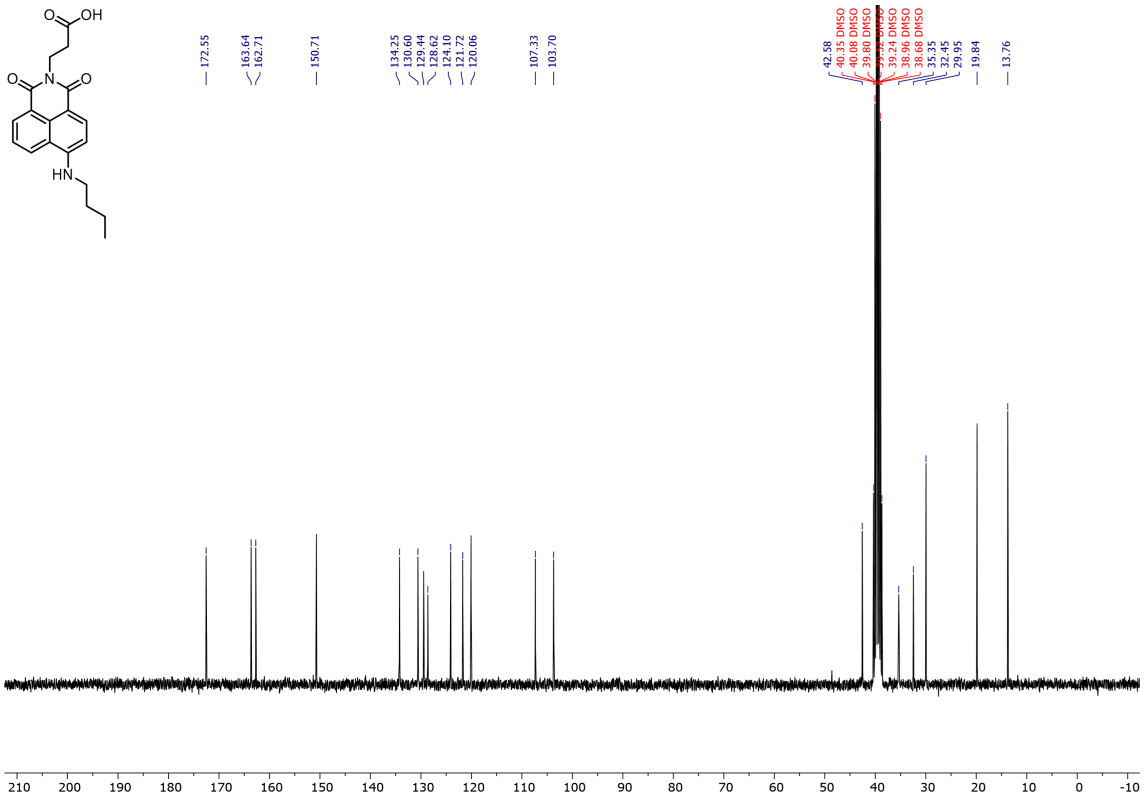
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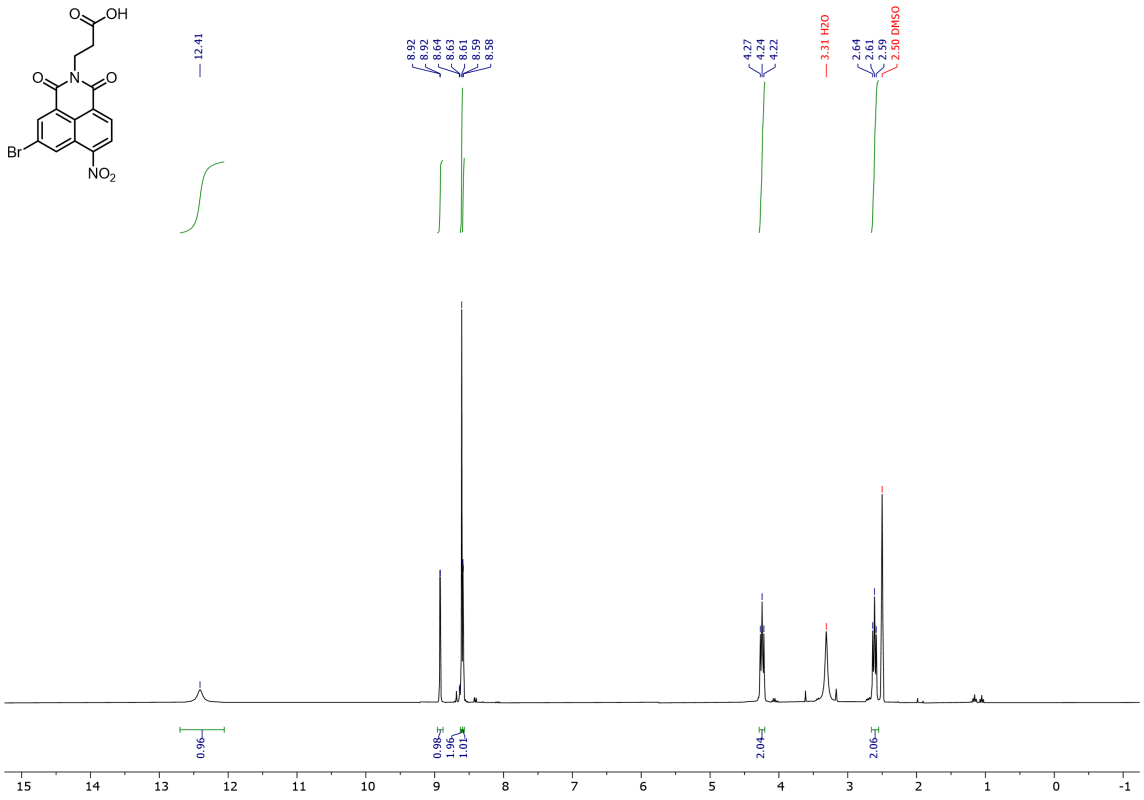
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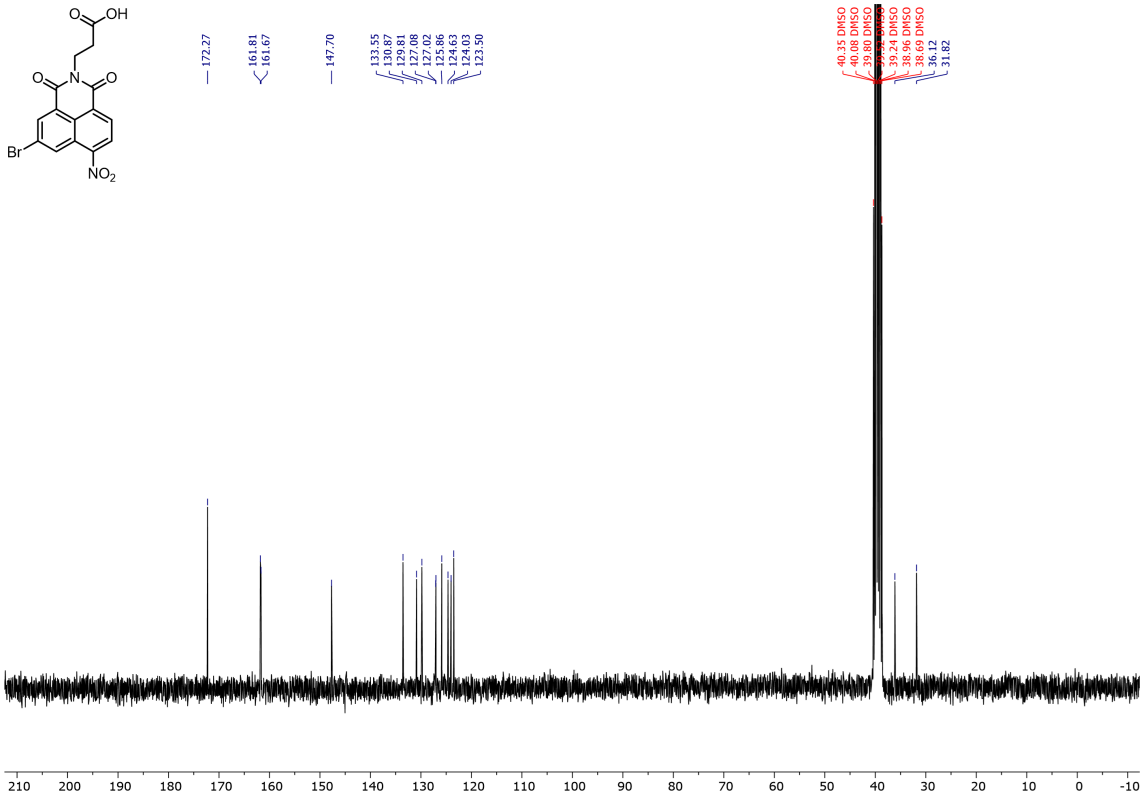
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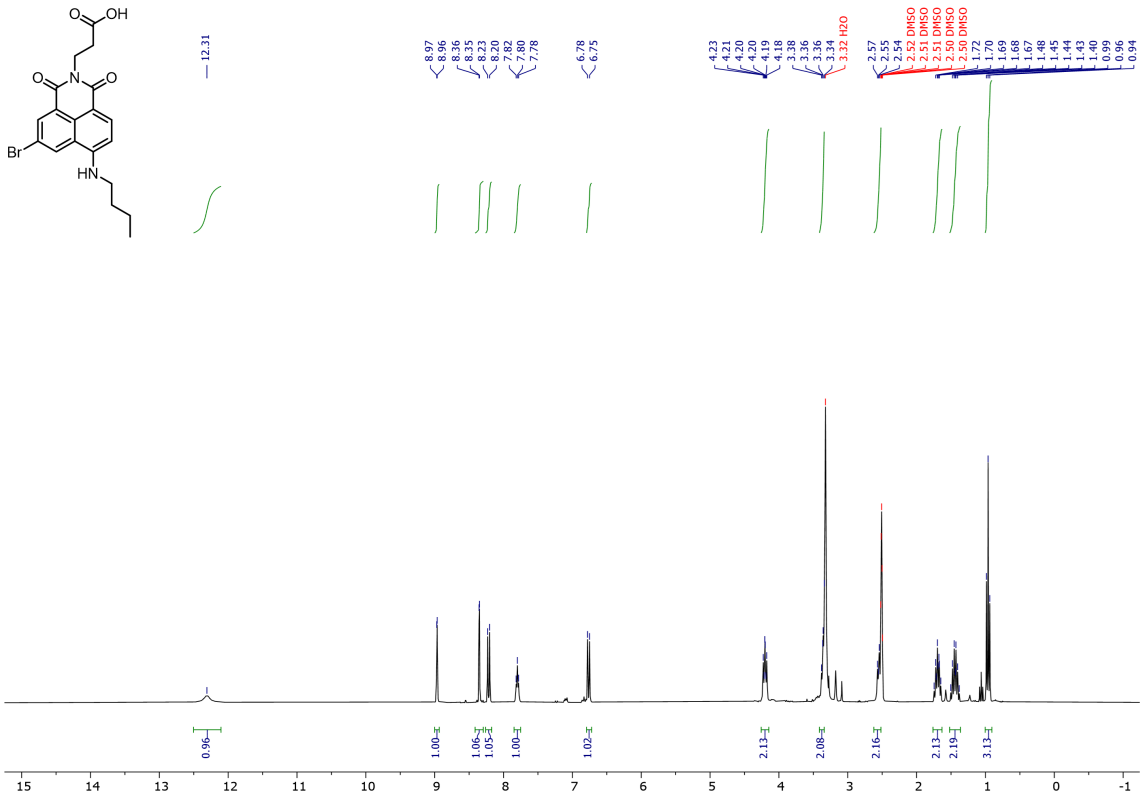
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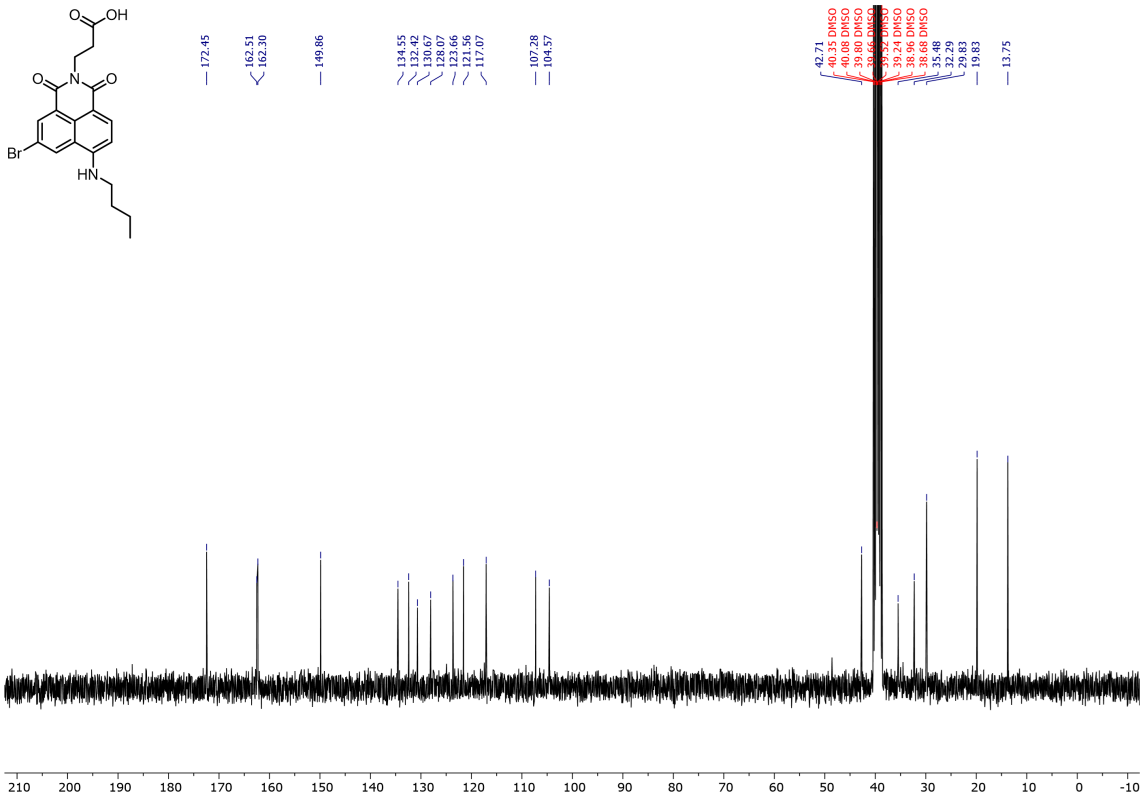
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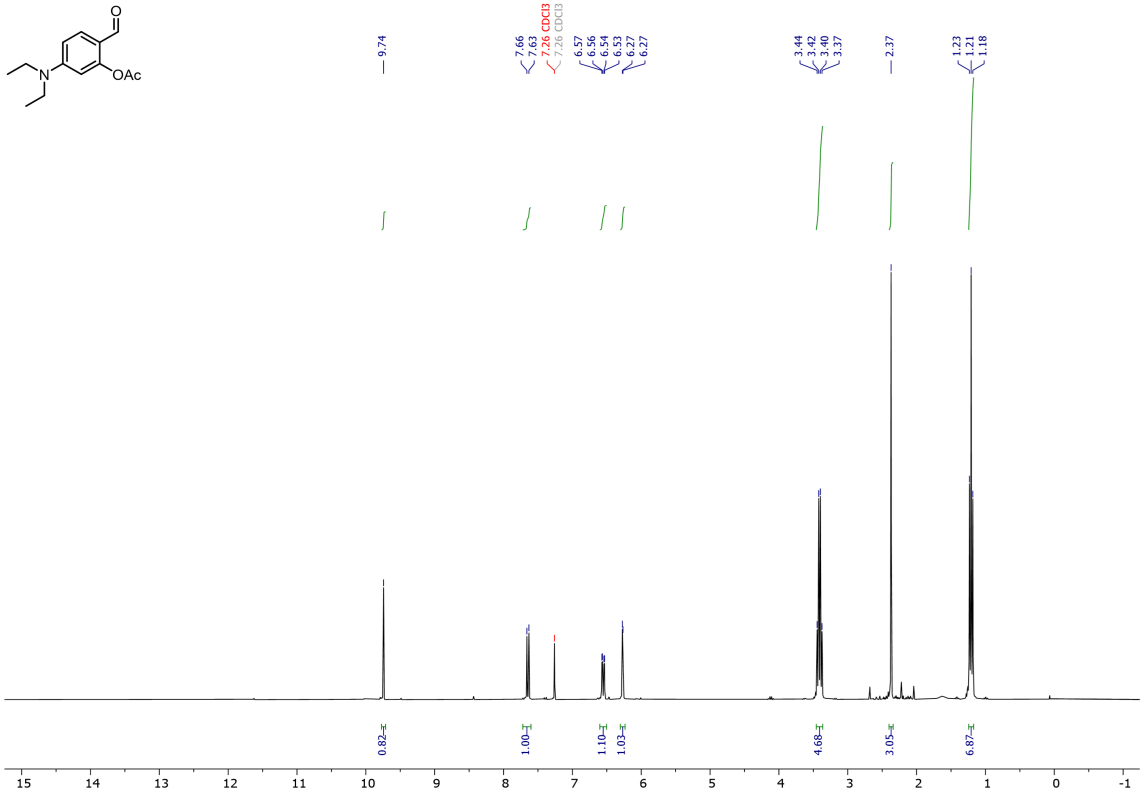
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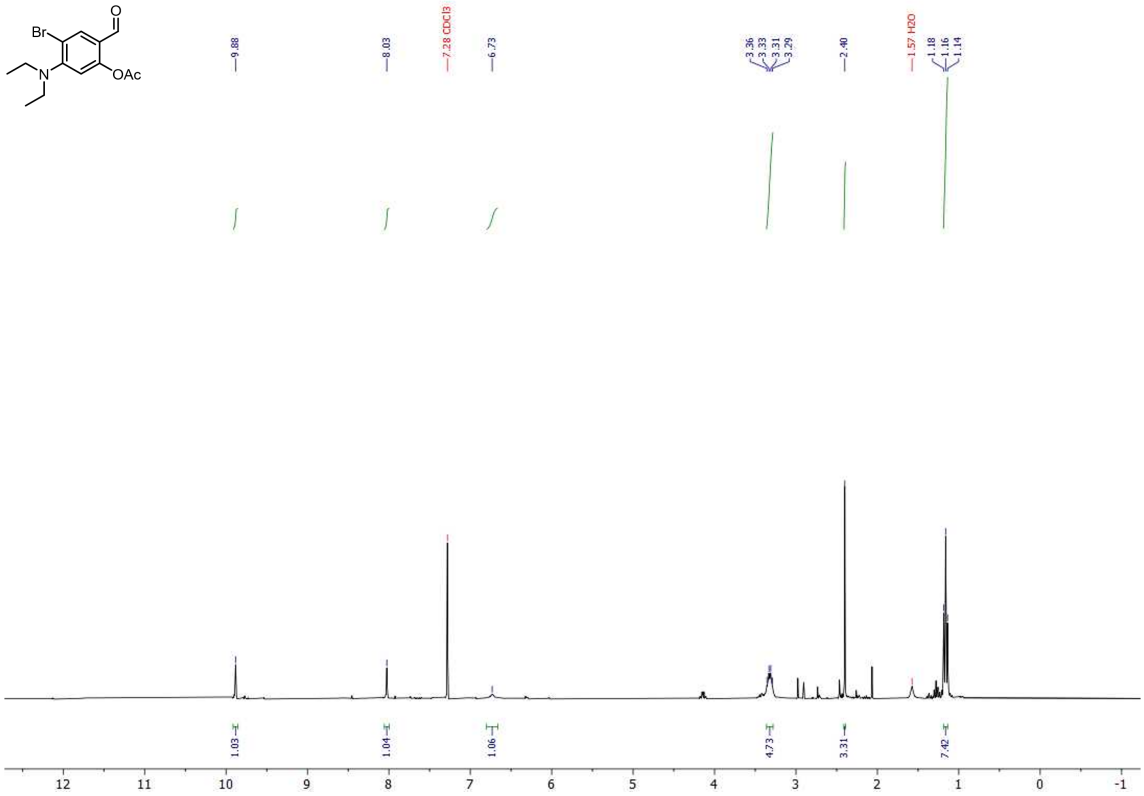
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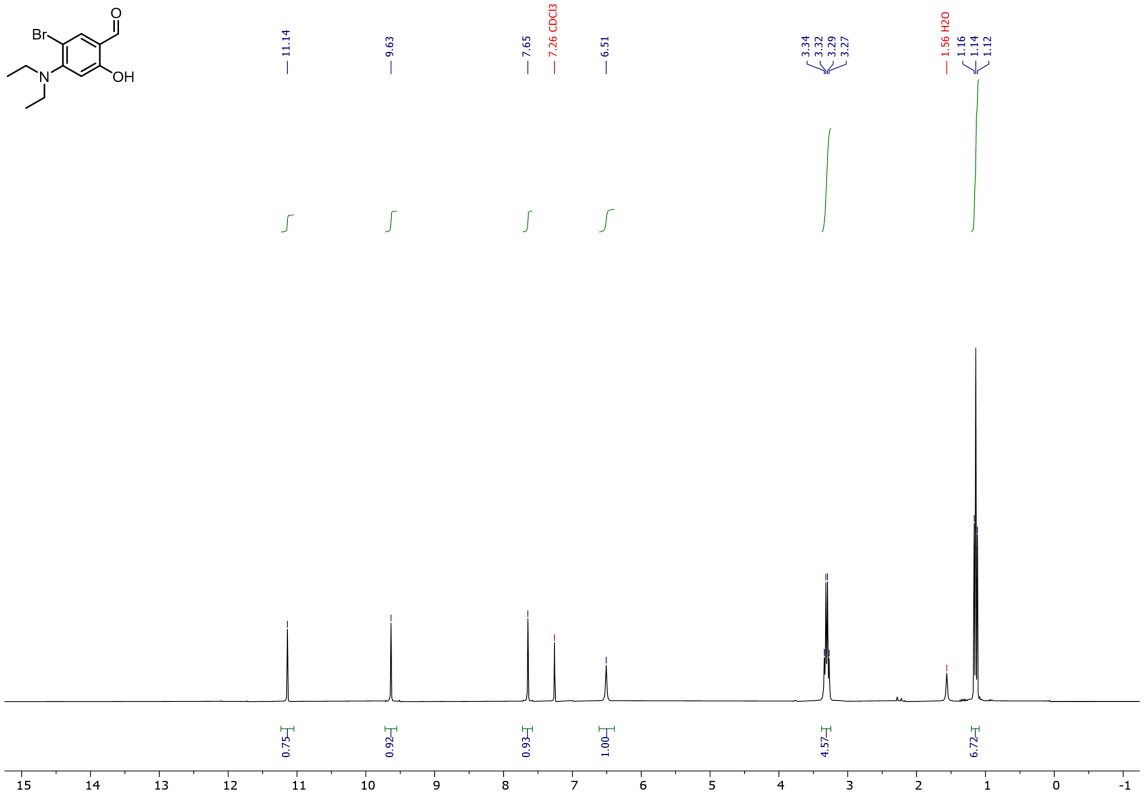
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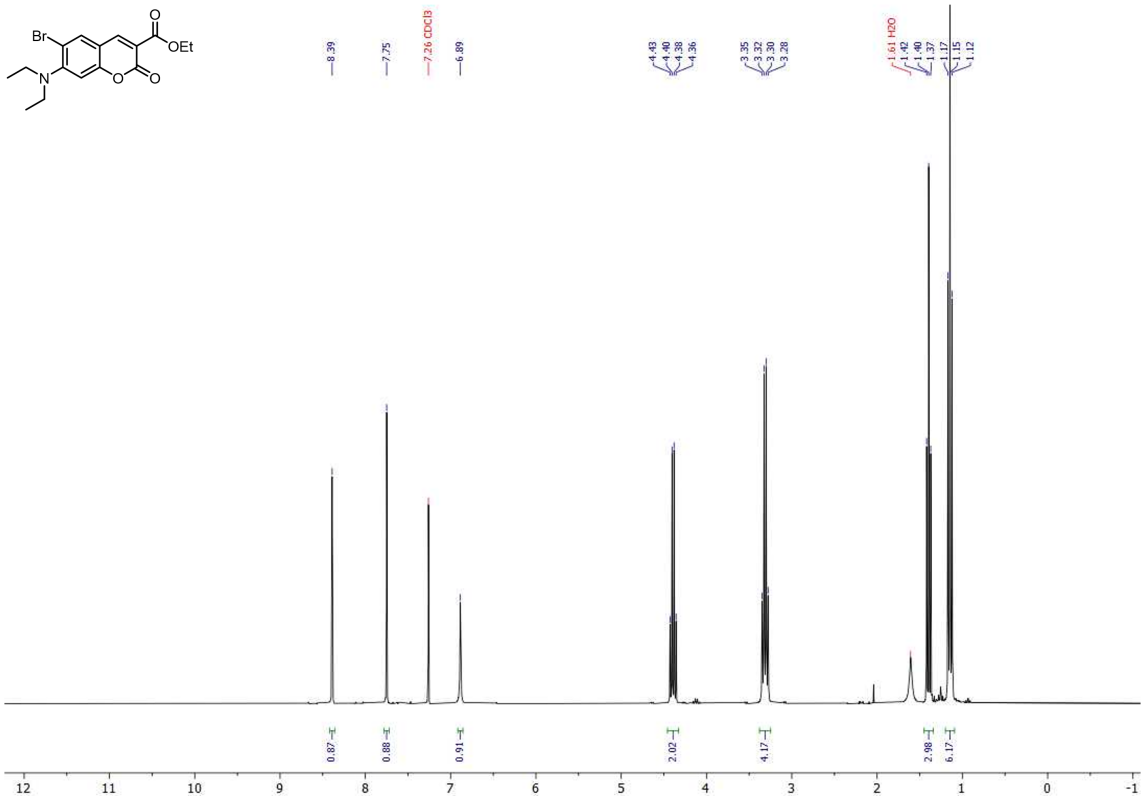
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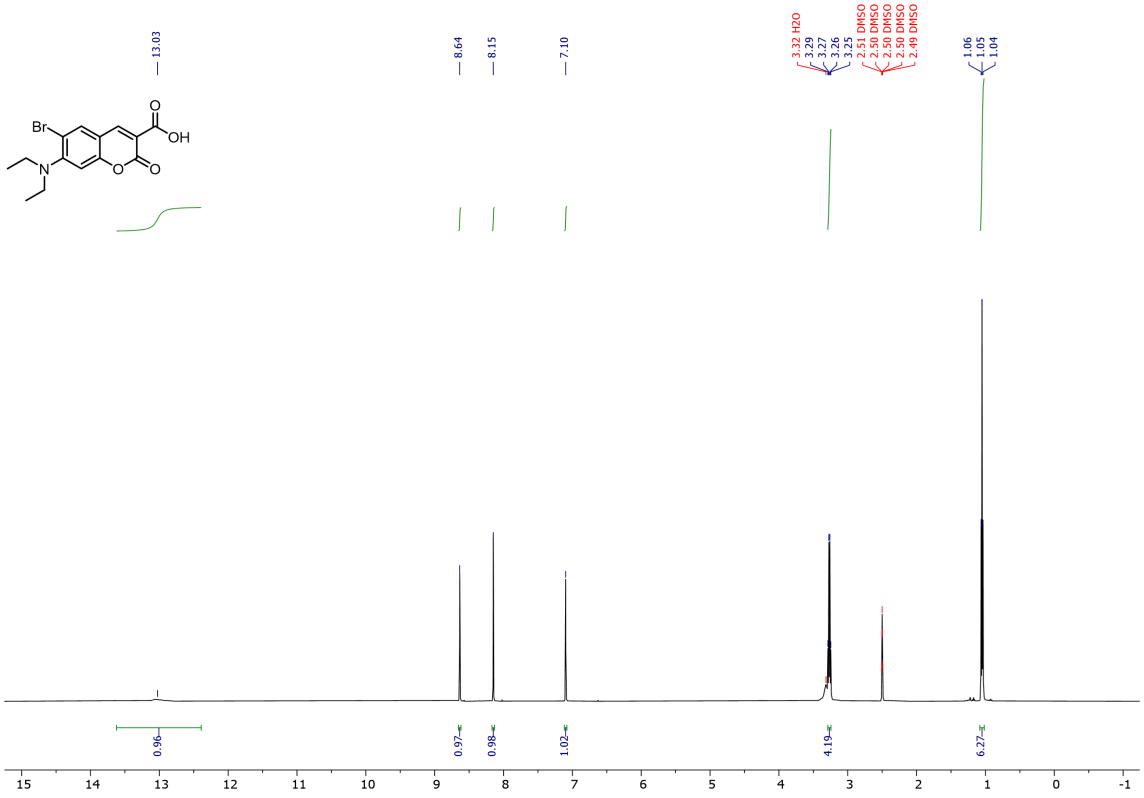
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1.05
1.04
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OH
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0.97
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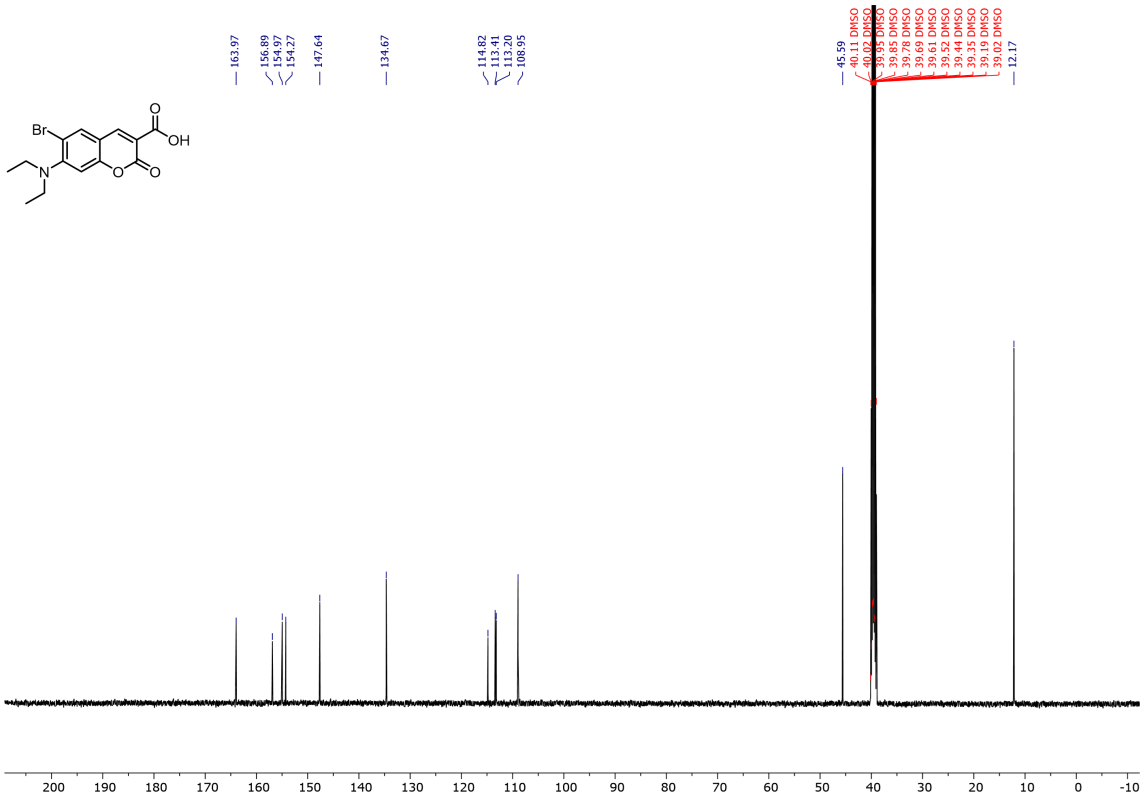
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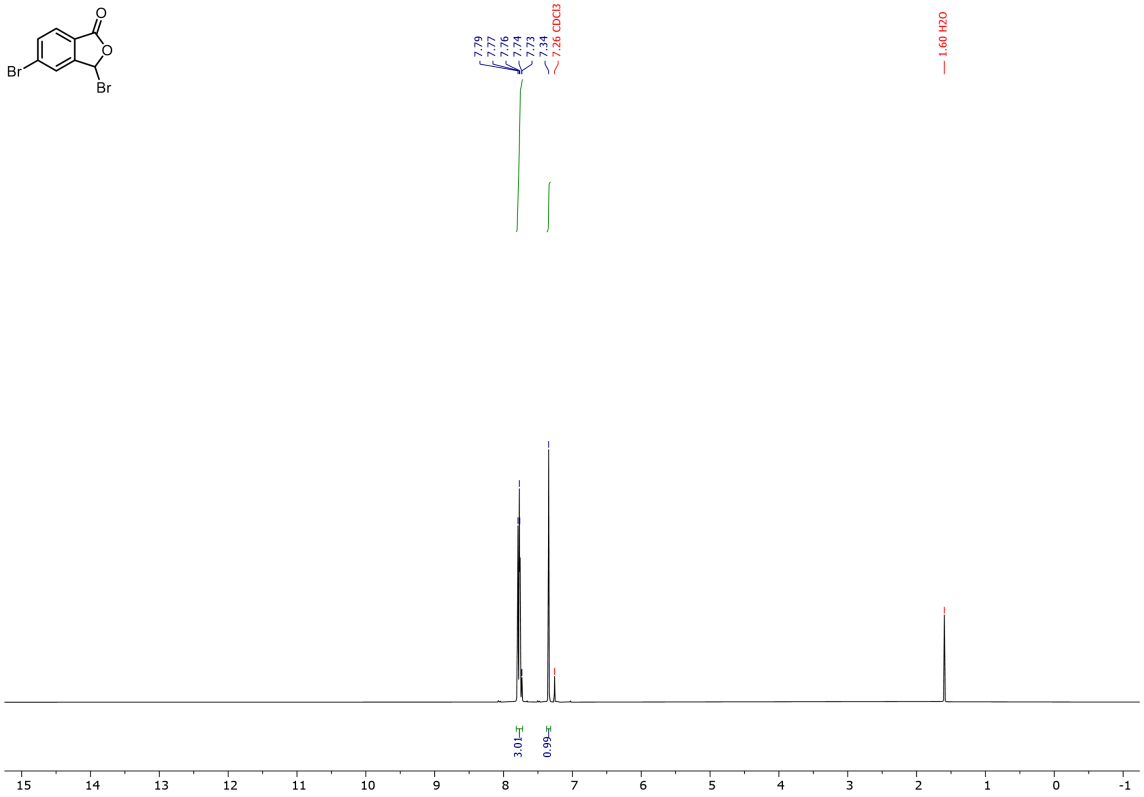
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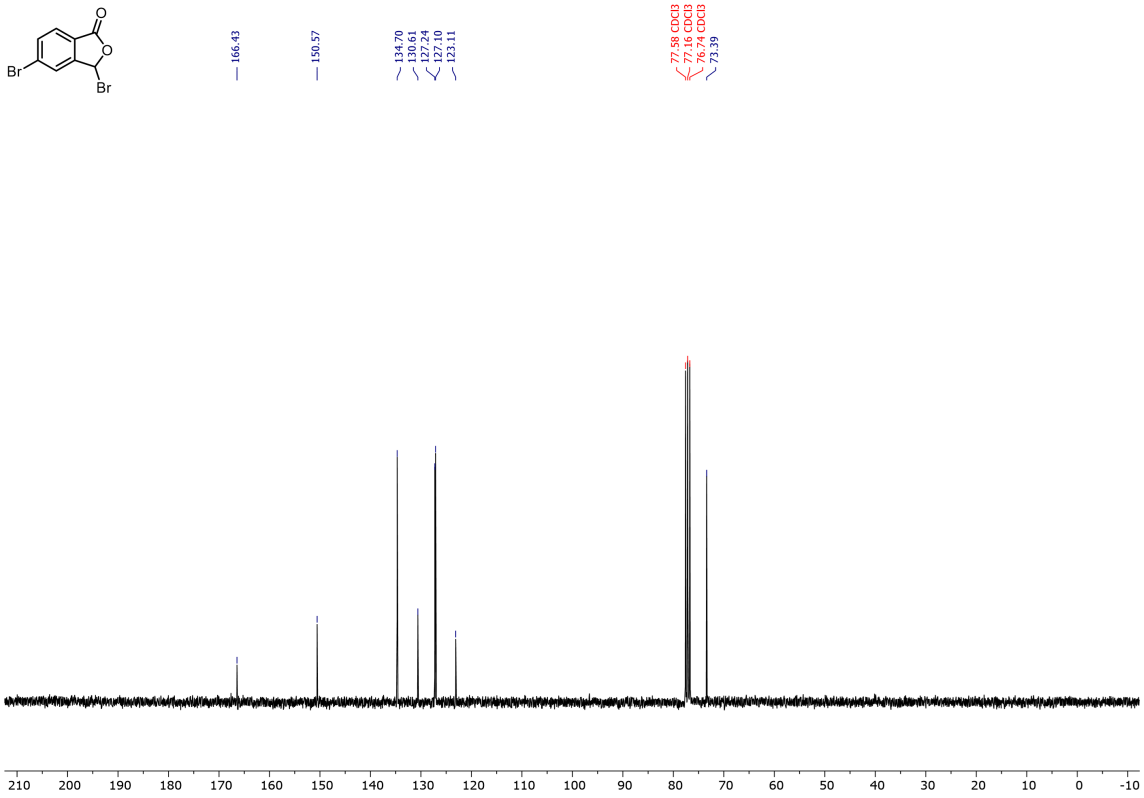
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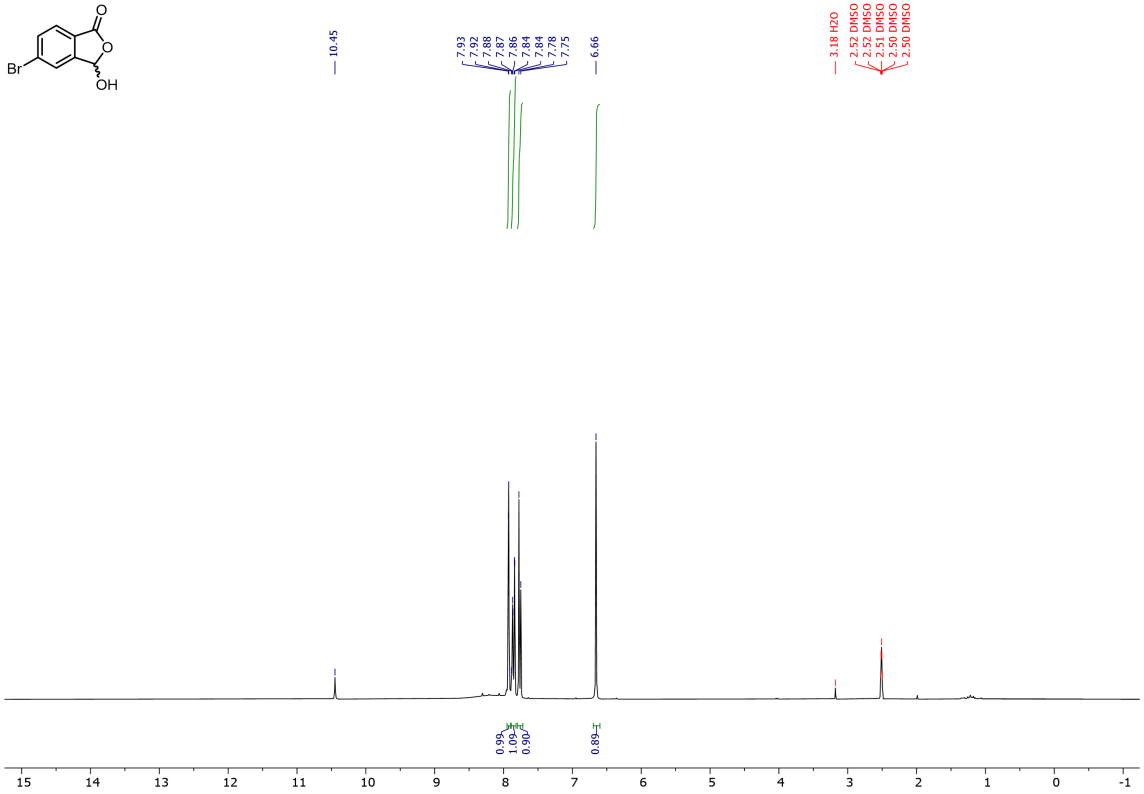
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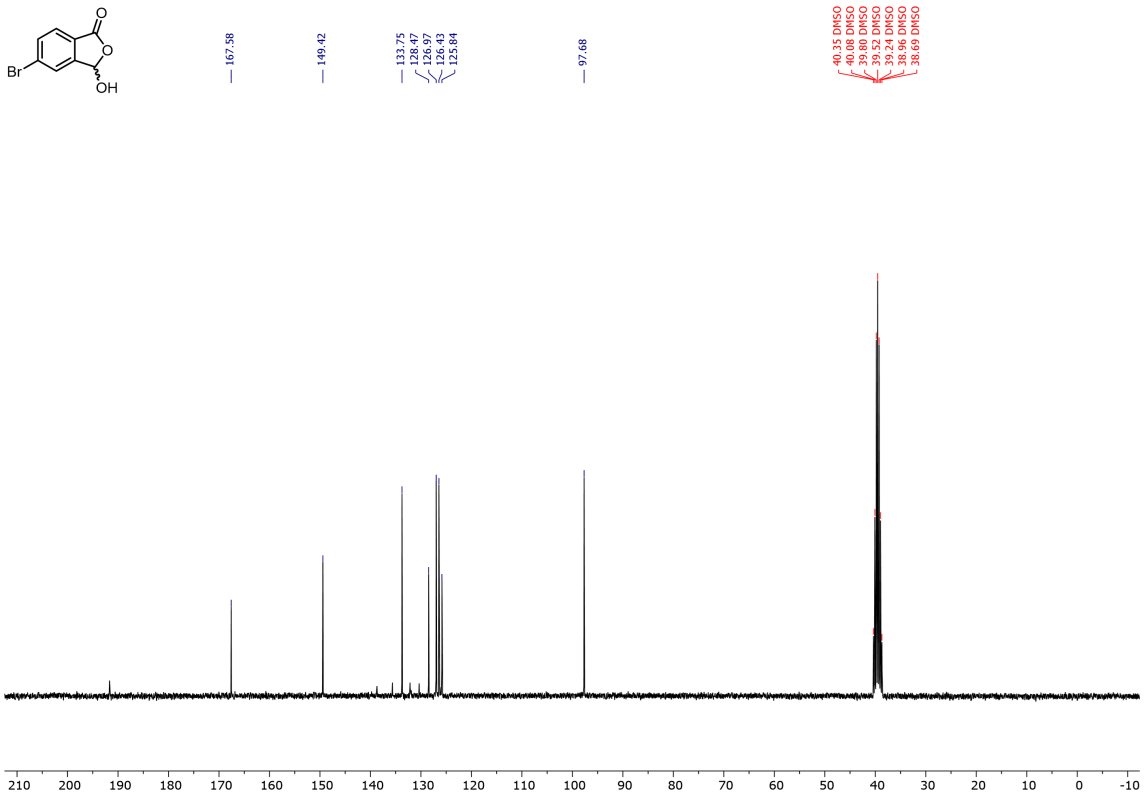
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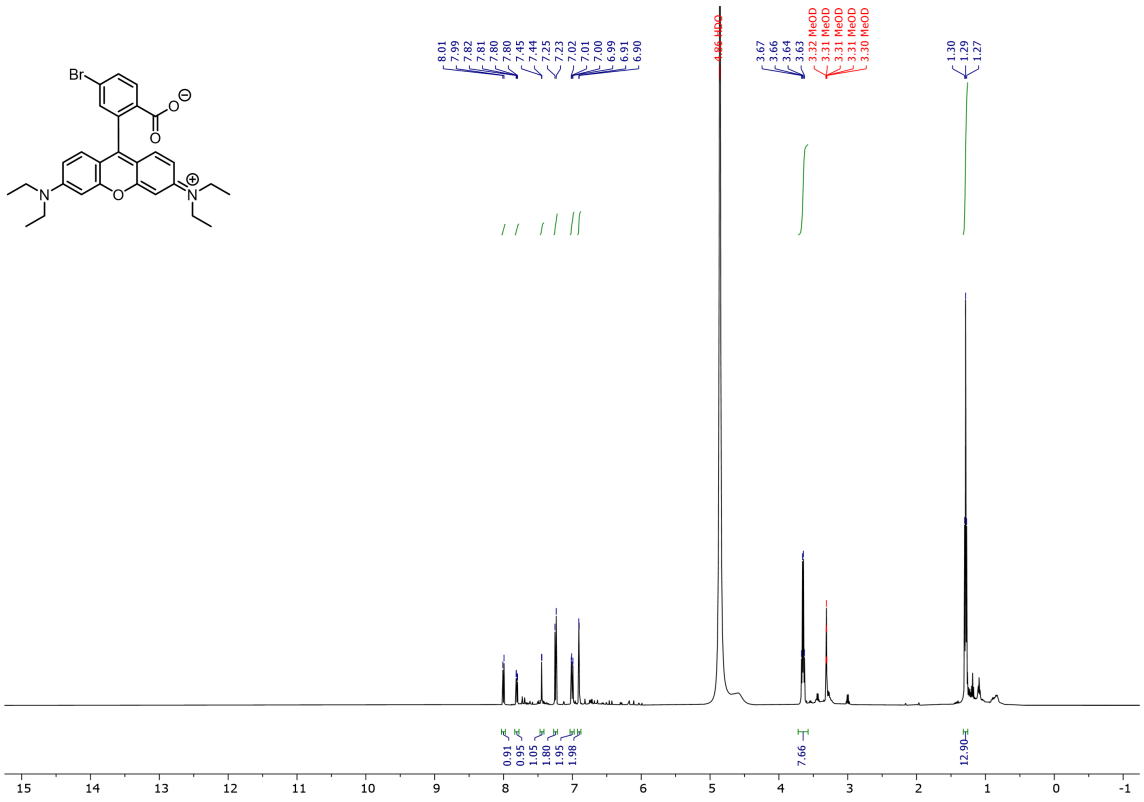
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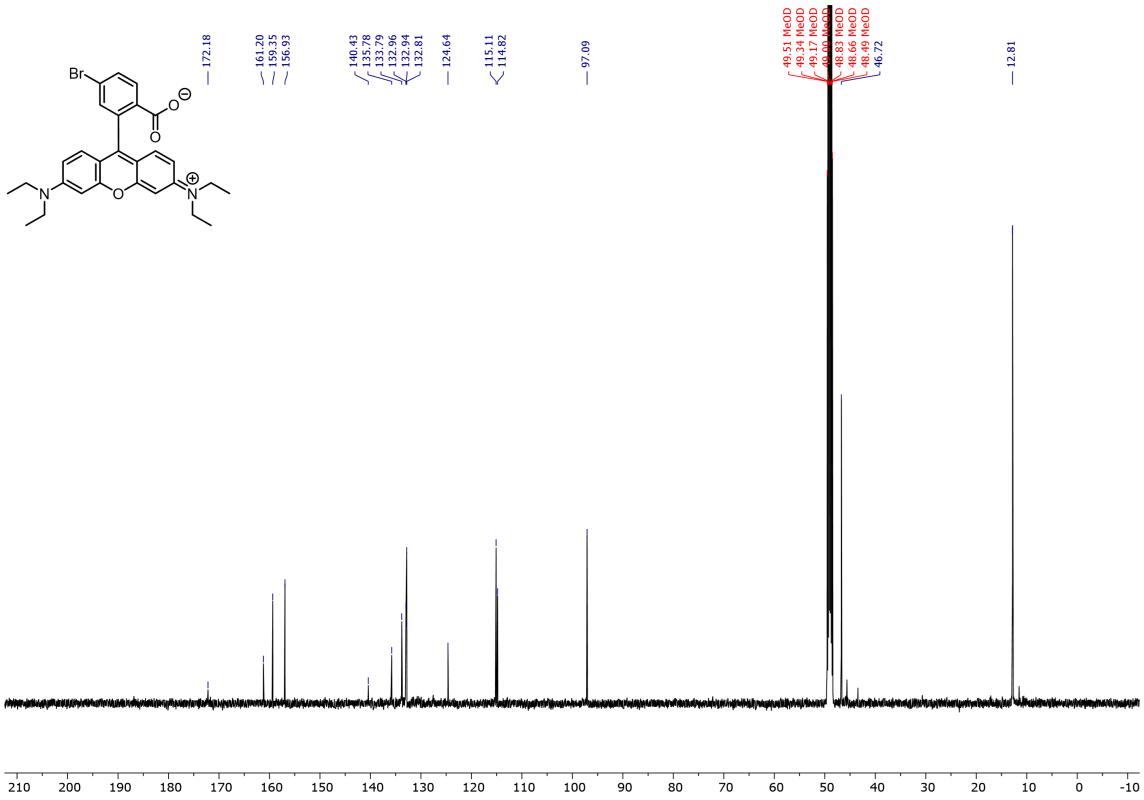
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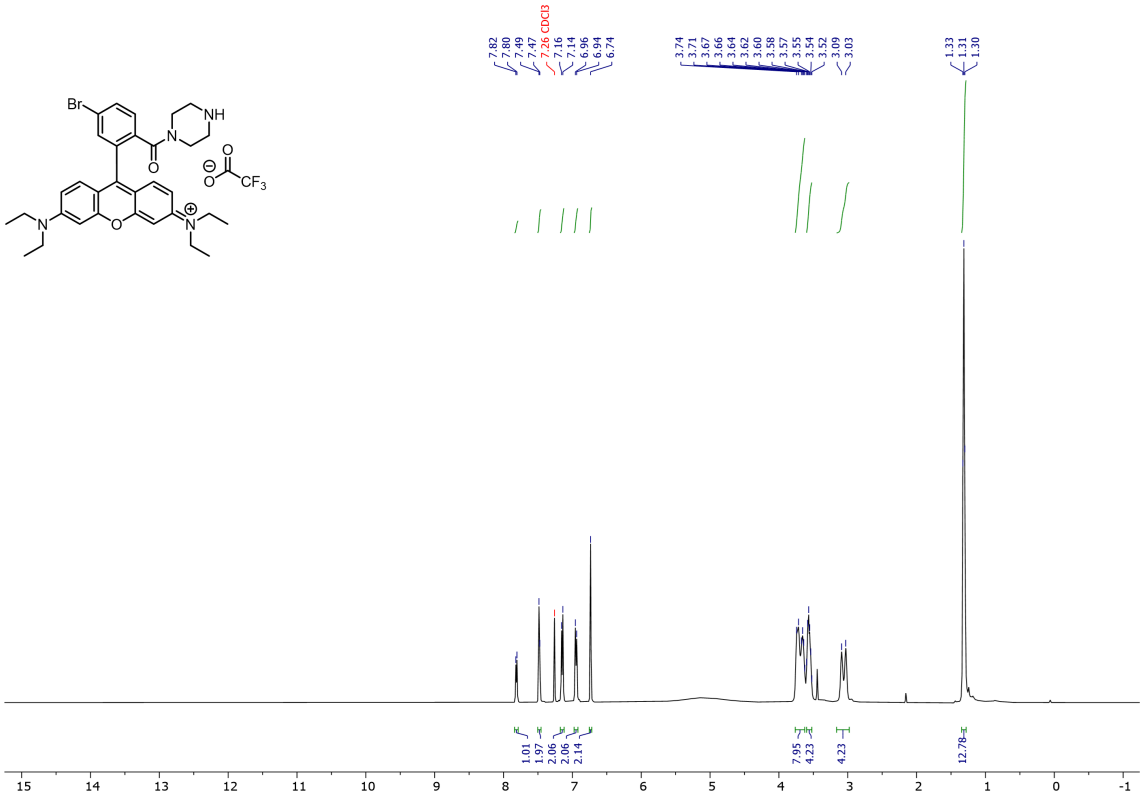
12.81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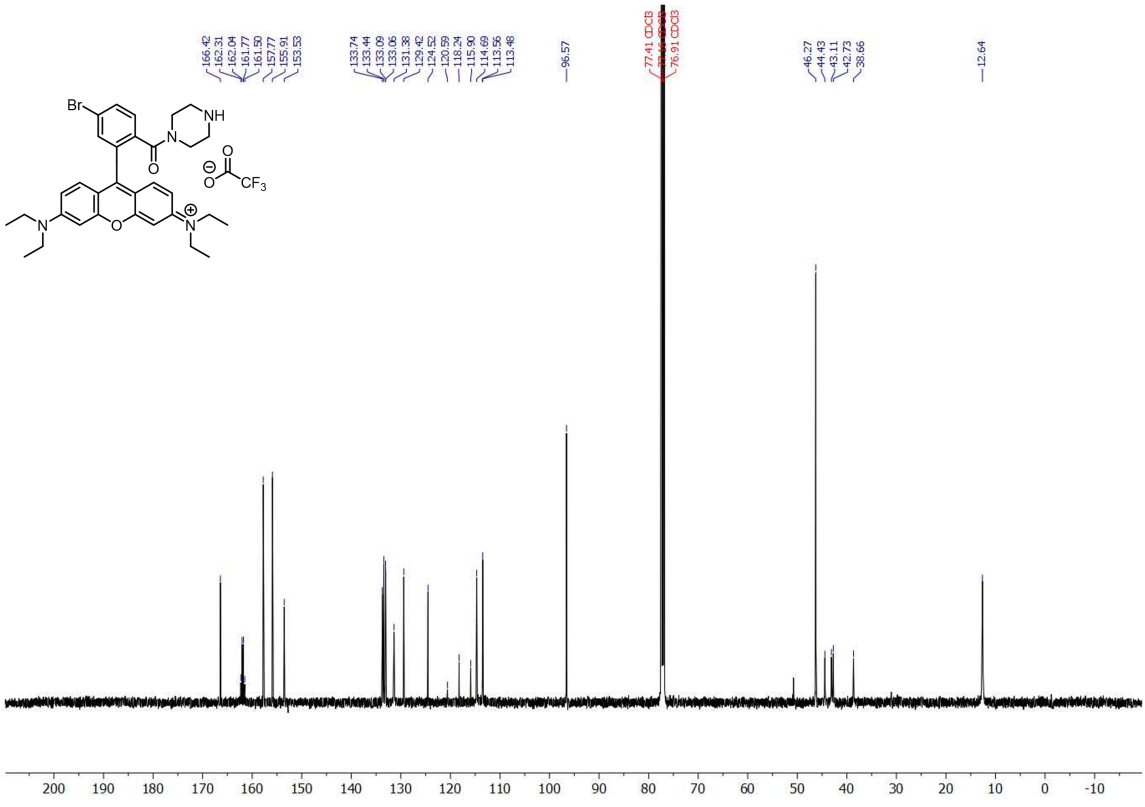
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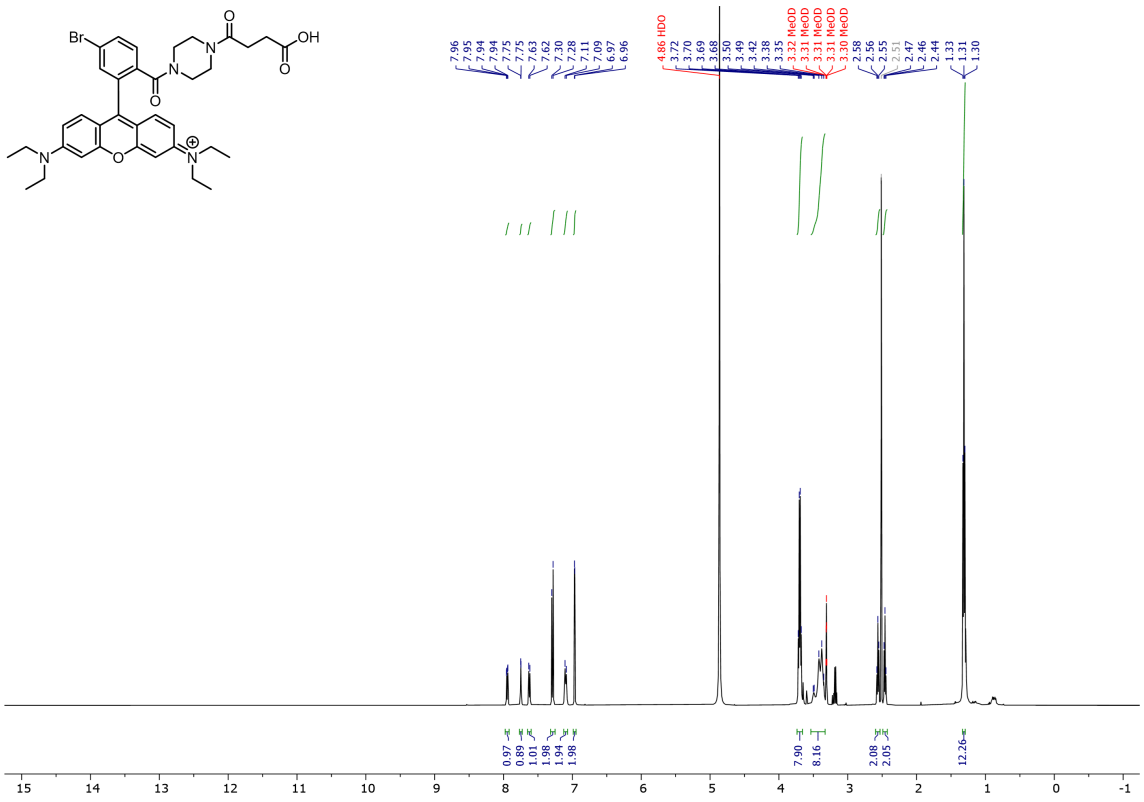


Photophysical properties of fluorescent dyes

All reported absolute quantum yield values (Φ) were measured on a Fluorolog QM-75-11-C (Horiba) equipped with a QuantaPhi-2 integrating sphere (Horiba). Measurements were carried out in optically dilute samples ($A < 0.1$) in PBS pH 7.4 (1X, ThermoFisher) or spectroscopic grade EtOH. Reported values are averages ($n = 3$).

Table S1. Photophysical properties of fluorescent dyes used in this study.

	Solvent	**λ_{abs} (nm)**	**λ_{em} (nm)**	**Φ_{fluo}**
Cou	EtOH	378	444	0.49
	PBS (pH 7.4)	405	468	<0.01[a]
CouBr	EtOH	376	436	0.07
	PBS (pH 7.4)	379	448	<0.01[a]
Np	EtOH	438	526	0.64
	PBS (pH 7.4)	450	552	0.23
NpBr	EtOH	448	542	0.34
	PBS (pH 7.4)	458	572	0.03
RhB	EtOH	542	573	0.49
	PBS (pH 7.4)	553	576	0.29
RhBBr	EtOH	567	589	0.35
	PBS (pH 7.4)	570	594	0.18

[a] below limit of detection

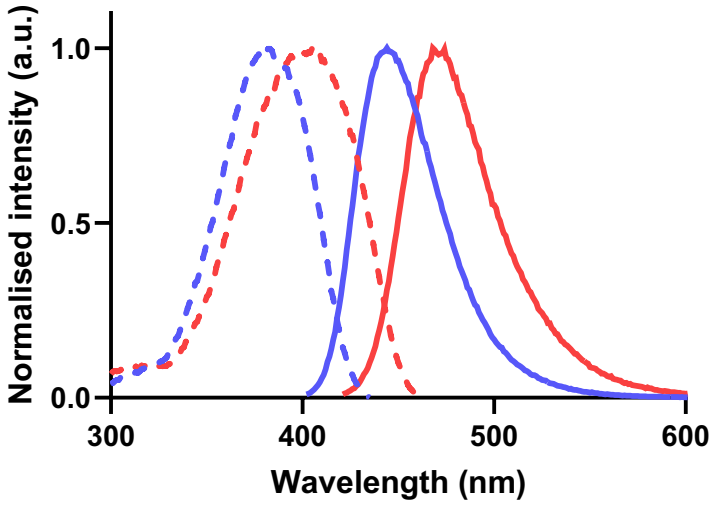


Figure S1. Normalised excitation (dashed) and emission (solid) spectra of **Cou** in EtOH (blue) and PBS pH 7.4 (red).

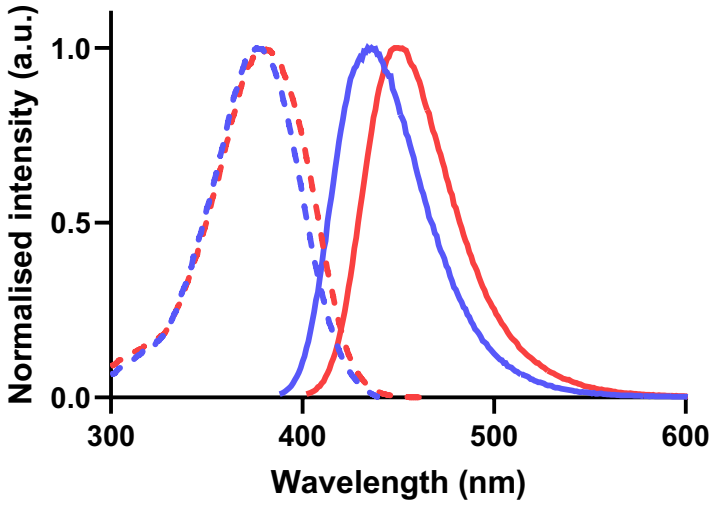


Figure S2. Normalised excitation (dashed) and emission (solid) spectra of **CouBr** in EtOH (blue) and PBS pH 7.4 (red).

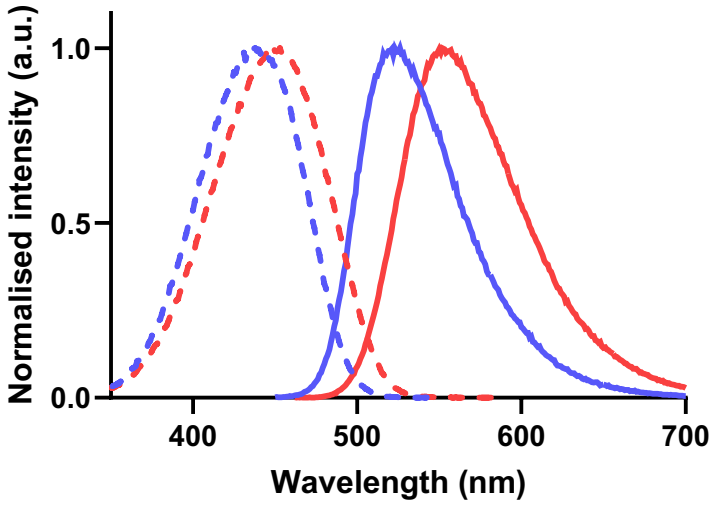


Figure S3. Normalised excitation (dashed) and emission (solid) spectra of **Np** in EtOH (blue) and PBS pH 7.4 (red).

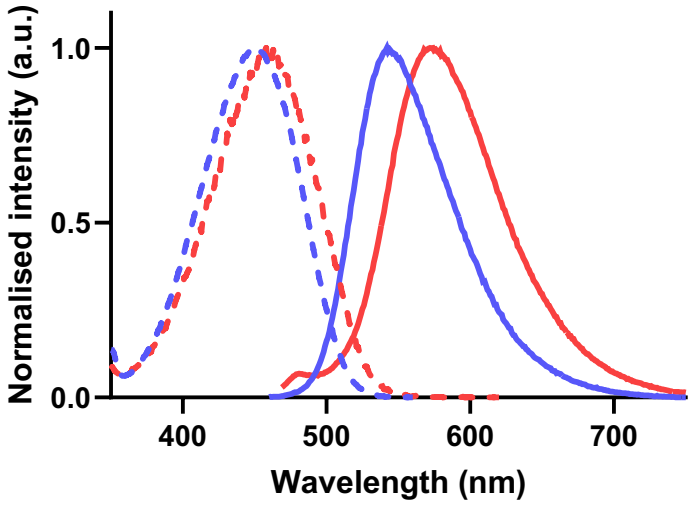


Figure S4. Normalised excitation (dashed) and emission (solid) spectra of **NpBr** in EtOH (blue) and PBS pH 7.4 (red).

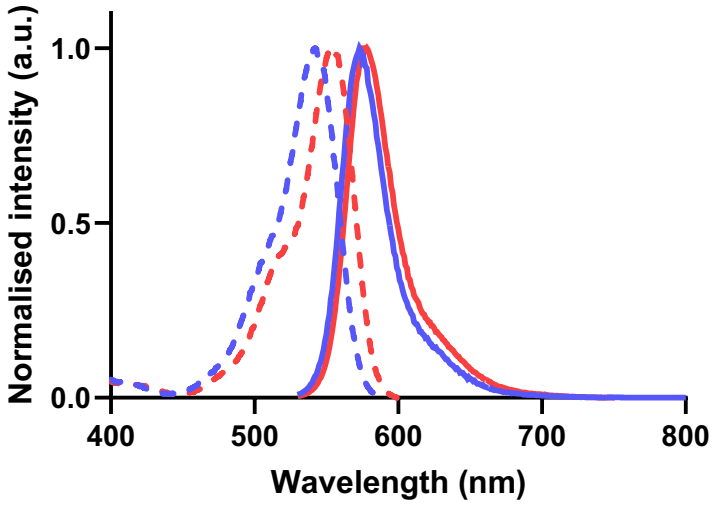


Figure S5. Normalised excitation (dashed) and emission (solid) spectra of **RhB** in EtOH (blue) and PBS pH 7.4 (red).

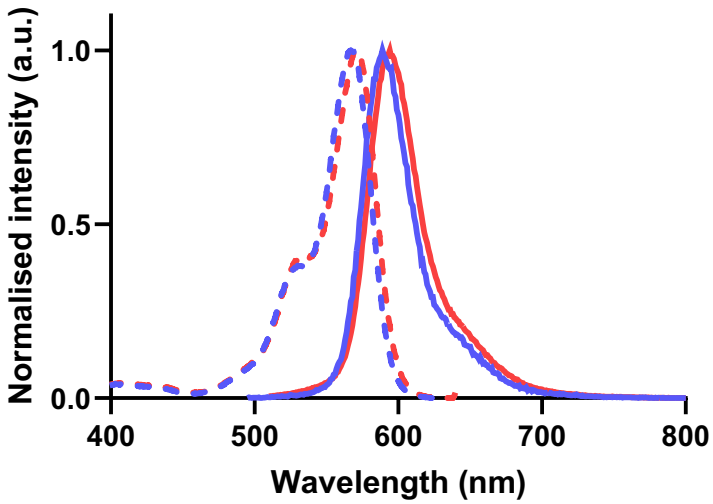


Figure S6. Normalised excitation (dashed) and emission (solid) spectra of **RhBBr** in EtOH (blue) and PBS pH 7.4 (red).

Analytical data of bismuth peptides

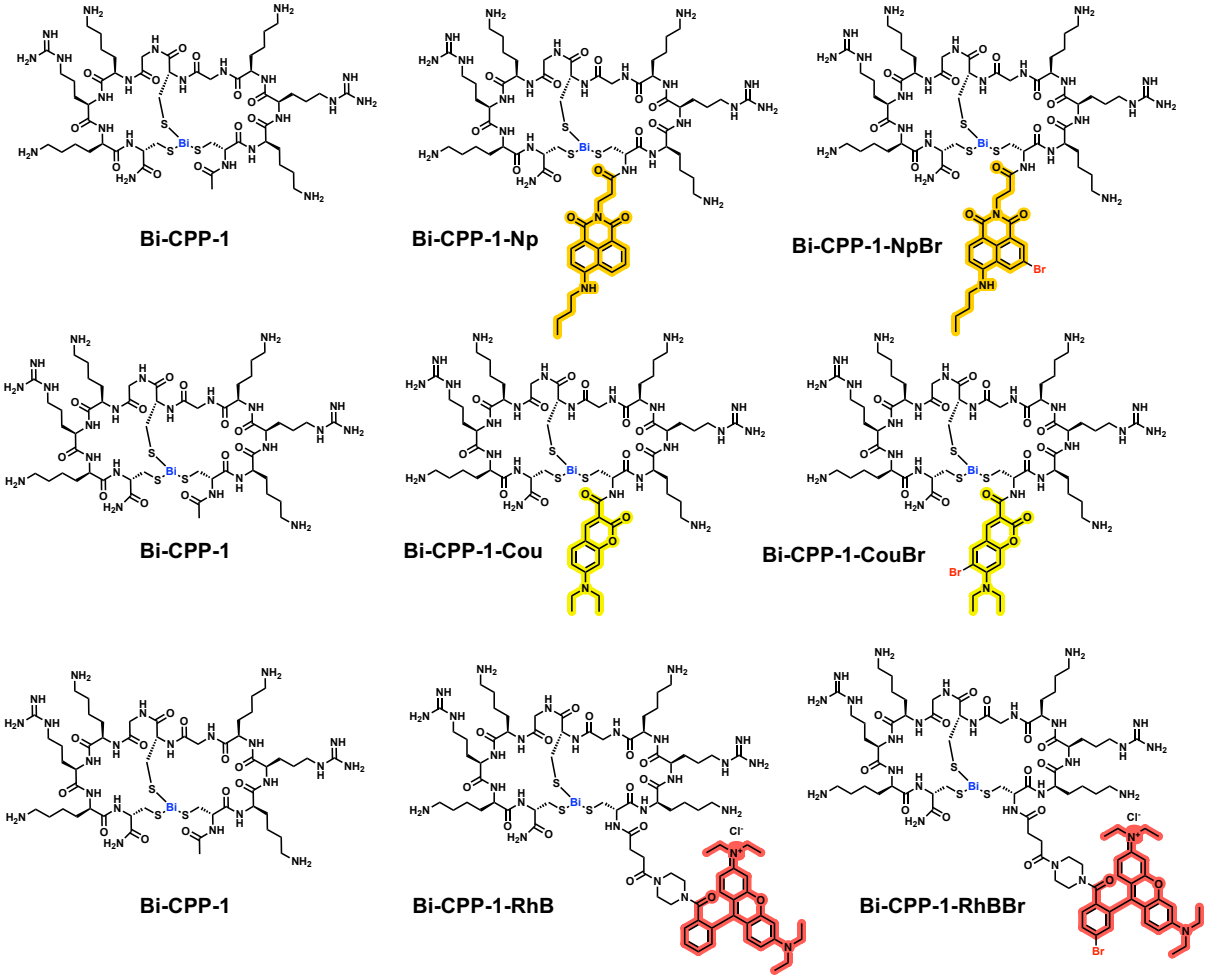


Figure S7. Chemical structures of investigated peptides based on **Bi-CPP-1**.

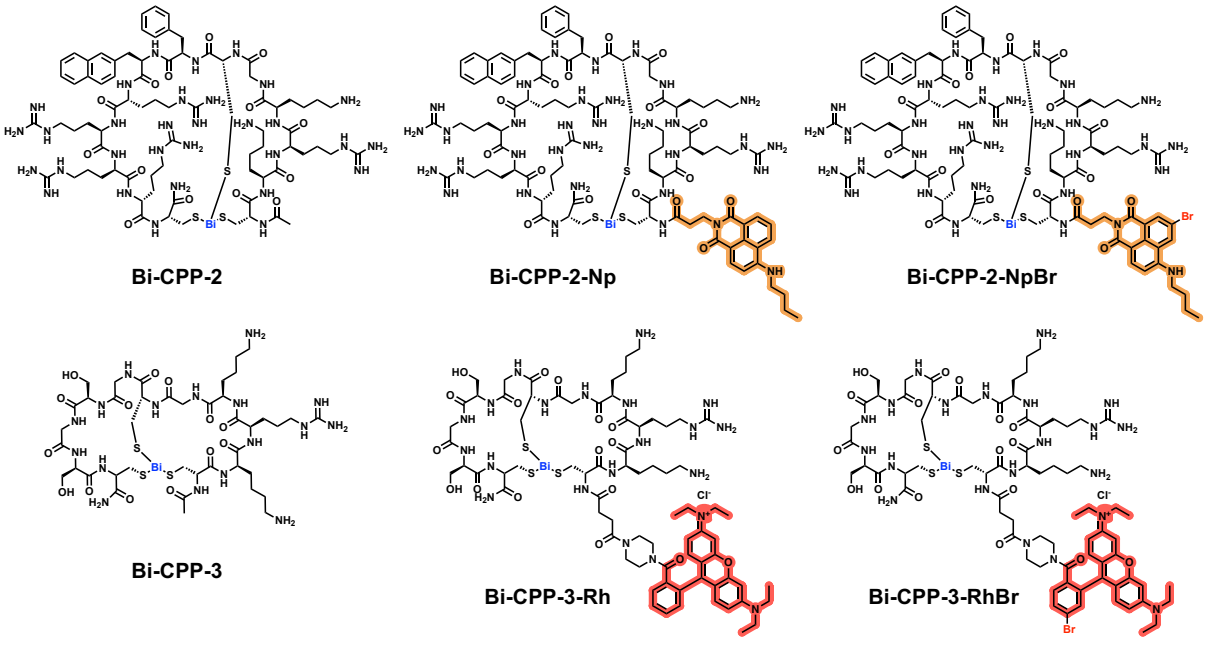


Figure S8. Chemical structures of investigated peptides based on **Bi-CPP-2** and **Bi-CPP-3**.

Table S2. High-resolution mass spectrometry (HRMS) data of synthesised bismuth peptides.

Compound	Molecular formula	HRMS calculated (detected) $[M+2H]^{2+}$	HRMS calculated (detected) $[M+3H]^{3+}$	HRMS calculated (detected) $[M+4H]^{4+}$	HRMS calculated (detected) $[M+5H]^{5+}$	HRMS calculated (detected) $[M+6H]^{6+}$
Bi-CPP-1		757.3311	505.2234	379.1695	303.5371	
	$C_{51}H_{95}BiN_{22}O_{12}S_3$	(757.3343)	(505.2250)	(379.1703)	(303.5378)	
Bi-CPP-1-		897.3917	598.5971	449.1998	359.5614	
Np	$C_{68}H_{111}BiN_{24}O_{14}S_3$	(897.3957)	(598.5993)	(449.2010)	(359.5622)	
Bi-CPP-1-		936.3470	624.5673	468.6774	375.1435	
NpBr	$C_{68}H_{110}BiBrN_{24}O_{14}S_3$	(937.3503)	(635.2357)	(469.1785)	(375.5440)	
Bi-CPP-1-		857.8706	572.2497	429.4392		
Cou	$C_{63}H_{106}BiN_{23}O_{14}S_3$	(857.8719)	(572.2521)	(429.4419)		
Bi-CPP-1-		896.8259[a]	598.2199[a]	448.9169[a]		
CouBr	$C_{63}H_{105}BiBrN_{23}O_{14}S_3$	(897.8260)	(598.8887)	(449.4175)		
Bi-CPP-2				503.9806	403.3860	336.3230
	$C_{77}H_{124}BiN_{31}O_{14}S_3$			(504.2332)	(403.5876)	(336.4909)
Bi-CPP-2-			765.0119	574.0109	459.4103	383.0099
Np	$C_{94}H_{140}BiN_{33}O_{16}S_3$		(765.3491)	(574.2633)	(459.6116)	(383.1772)
Bi-CPP-2-			790.9821[a]	593.4885[a]	474.9924[a]	395.9950[a]
NpBr	$C_{94}H_{139}BiBrN_{33}O_{16}S_3$		(791.6521)	(593.9909)	(475.3940)	(396.3294)
Bi-CPP-3	$C_{41}H_{72}BiN_{17}O_{14}S_3$	666.7284 (666.7290)				

Compound	Molecular formula	HRMS calculated (detected) $[M+1H]^{2+}$	HRMS calculated (detected) $[M+2H]^{3+}$	HRMS calculated (detected) $[M+3H]^{4+}$	HRMS calculated (detected) $[M+4H]^{5+}$
Bi-CPP-1-			688.9906	516.9949	413.7975
RhB	$C_{85}H_{134}BiN_{26}O_{15}S_3^+$		(688.9905)	(516.9946)	(413.7965)
Bi-CPP-1-			714.6248[a]	536.2206[a]	429.1780[a]
RhBBr	$C_{85}H_{133}BiBrN_{26}O_{15}S_3^+$		(715.2910)	(536.7228)	(429.5777)
Bi-CPP-3-		942.3792	628.5887	471.6935	
RhB	$C_{75}H_{111}BiN_{21}O_{17}S_3^+$	(942.3802)	628.5934	471.6944	
Bi-CPP-3-		981.3344[a]	654.5589[a]	491.1711[a]	
RhBBr	$C_{75}H_{110}BiBrN_{21}O_{17}S_3^+$	(981.8325)	(654.8920)	(491.4201)	

[a]Atomic mass of ^{79}Br used for the calculation.

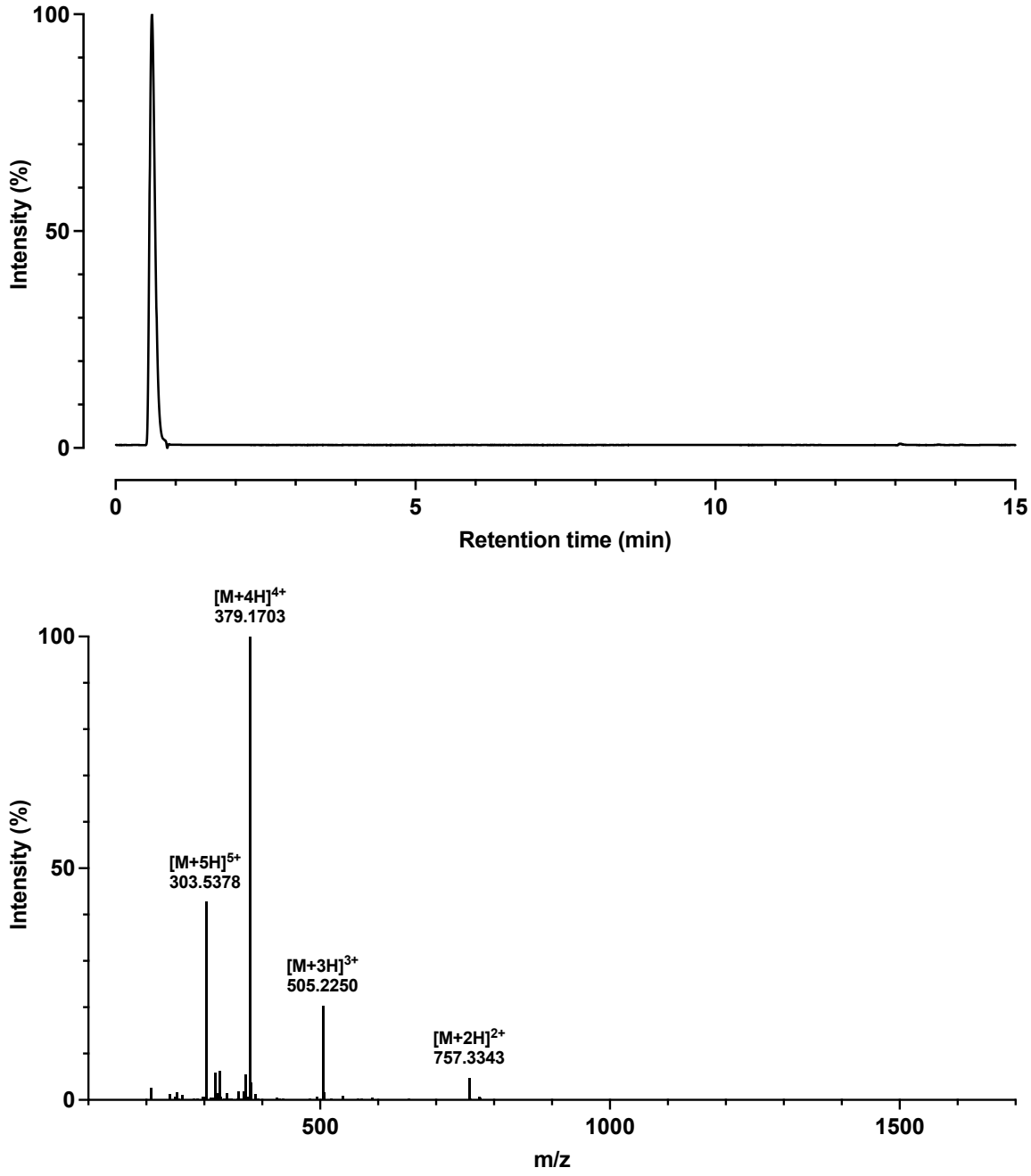


Figure S9. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-1** and its MS spectrum.

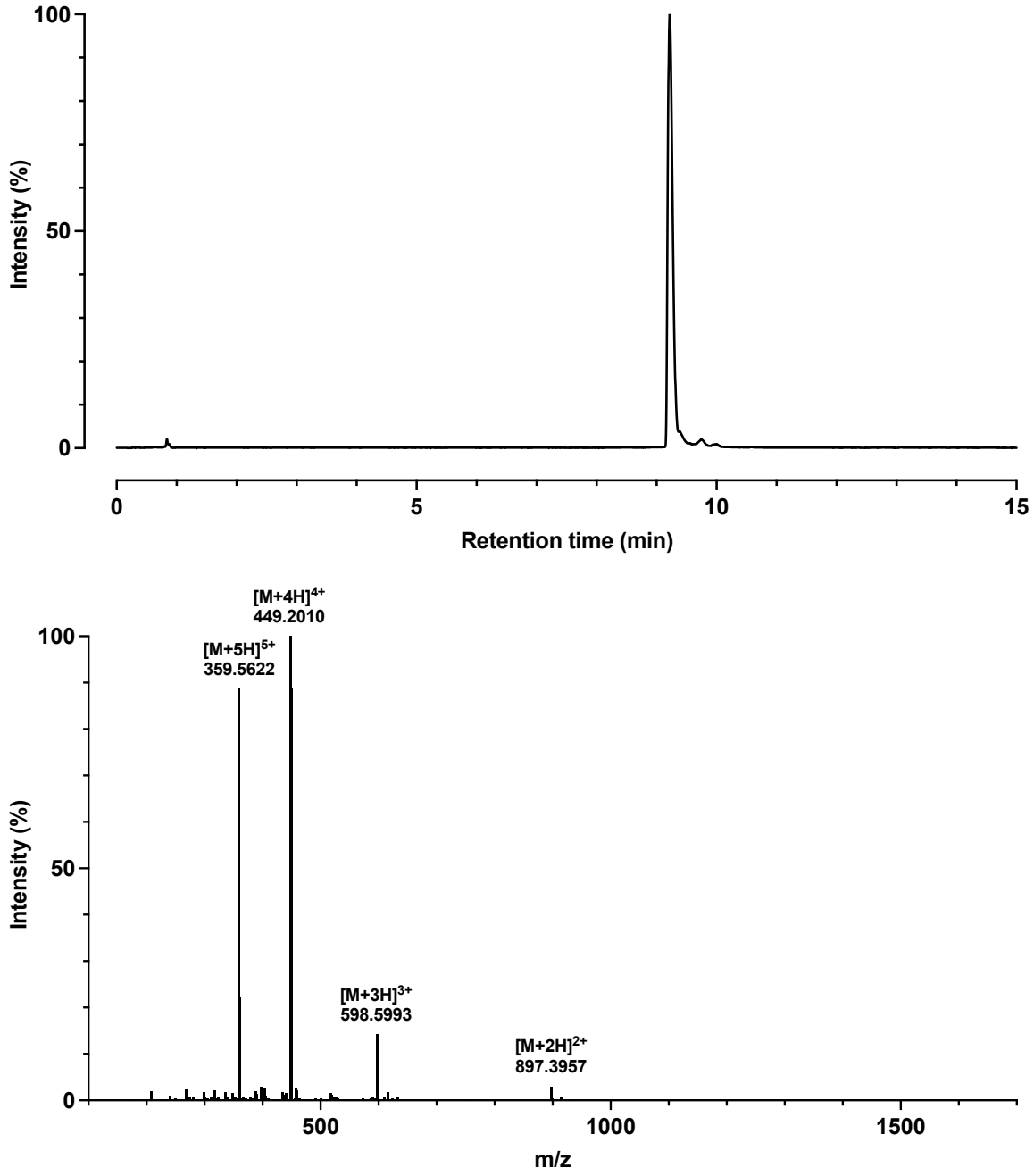


Figure S10. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-1-Np** and its MS spectrum.

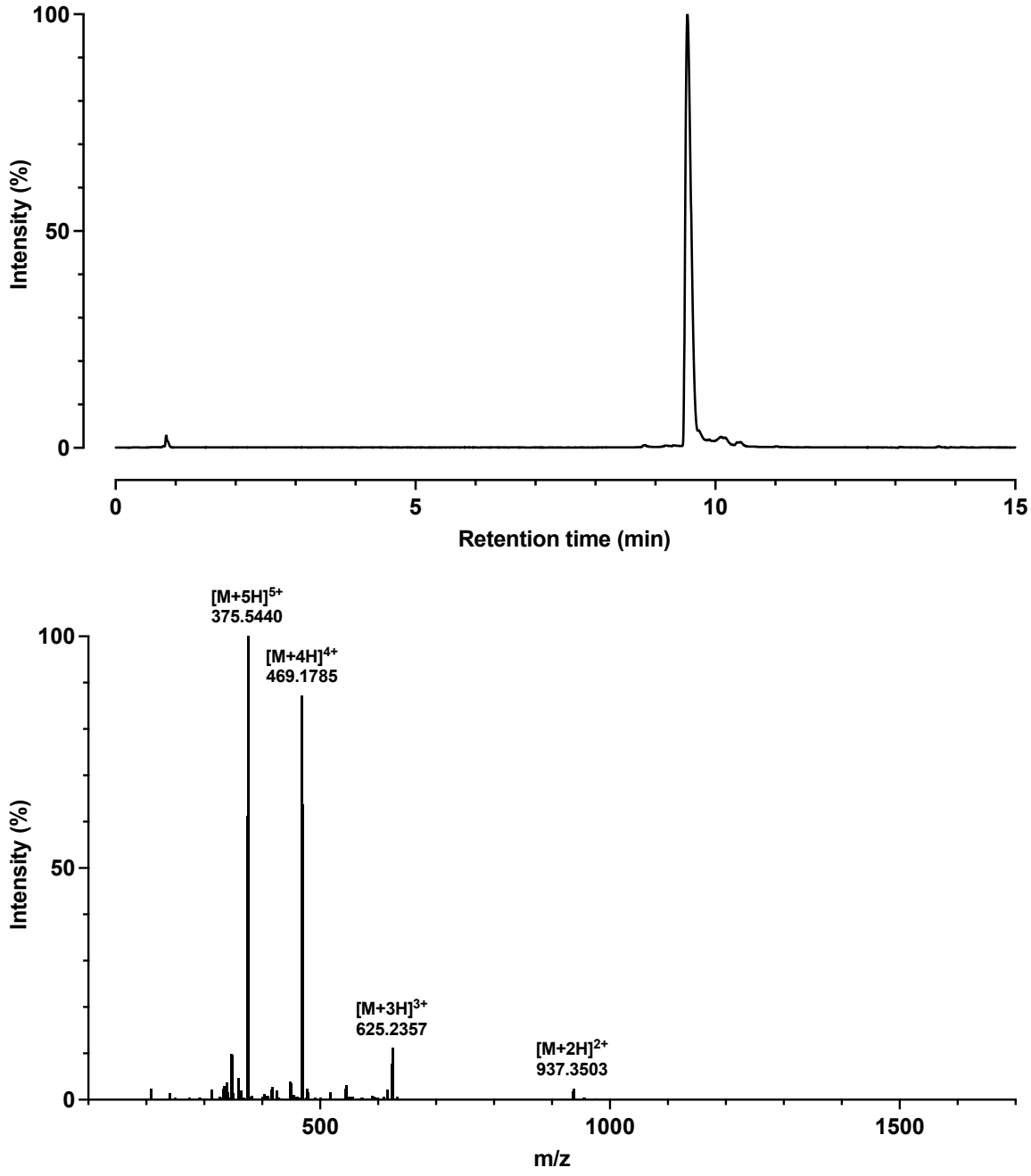


Figure S11. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-1-NpBr** and its MS spectrum.

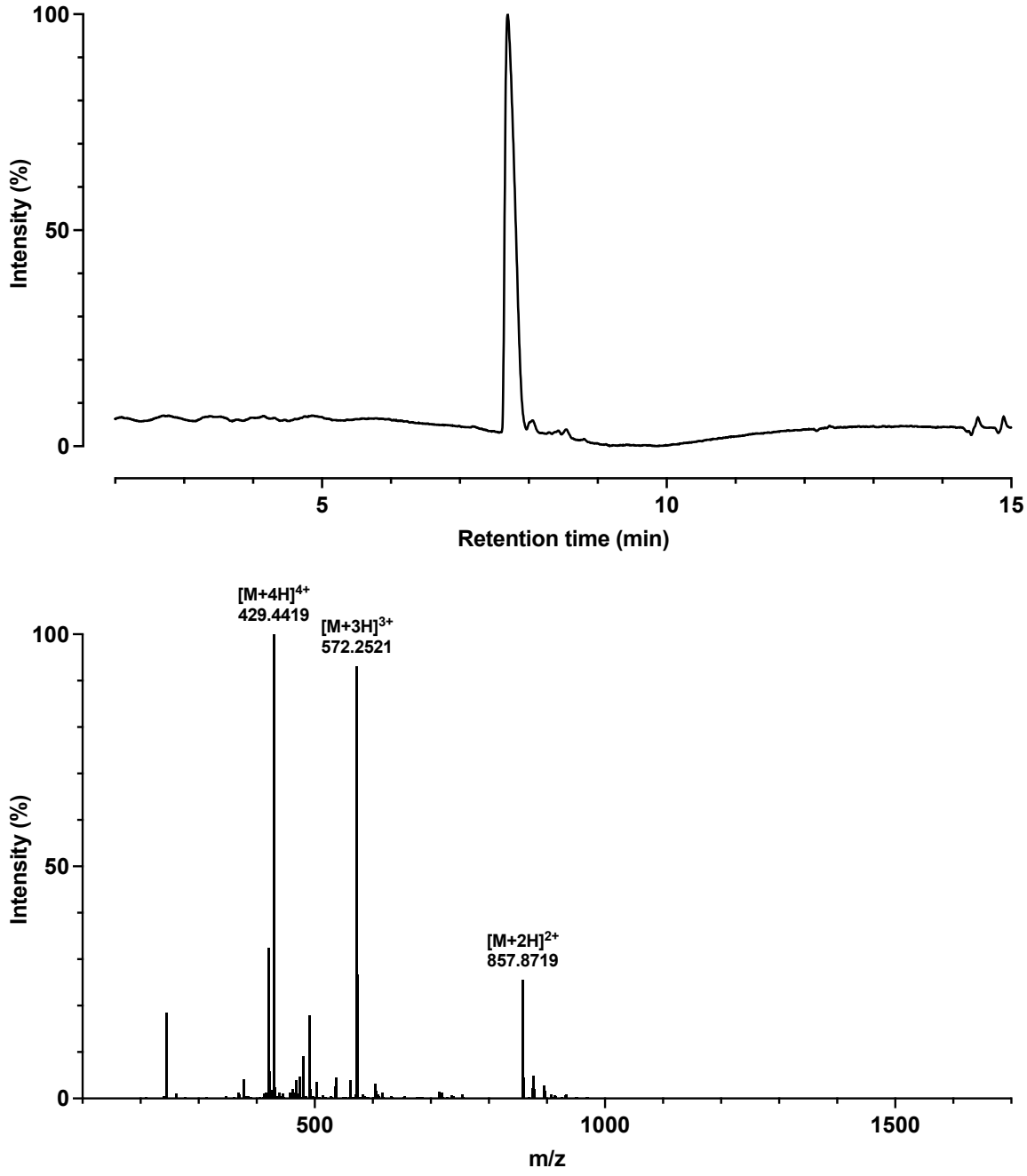


Figure S12. LC-MS chromatogram (214 nm, method A) of purified **Bi-CPP-1-Cou** and its MS spectrum.

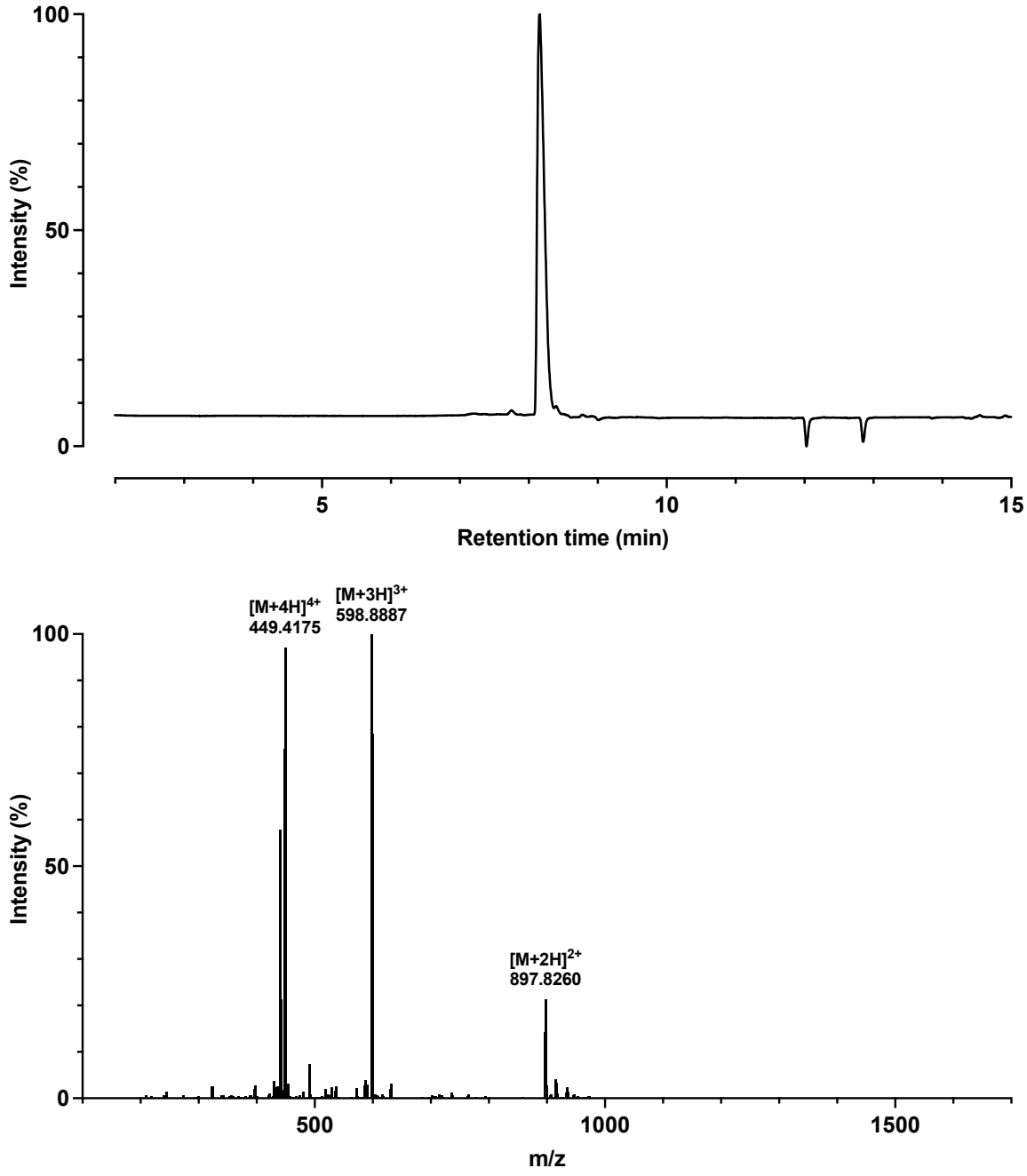


Figure S13. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-1-CouBr** and its MS spectrum.

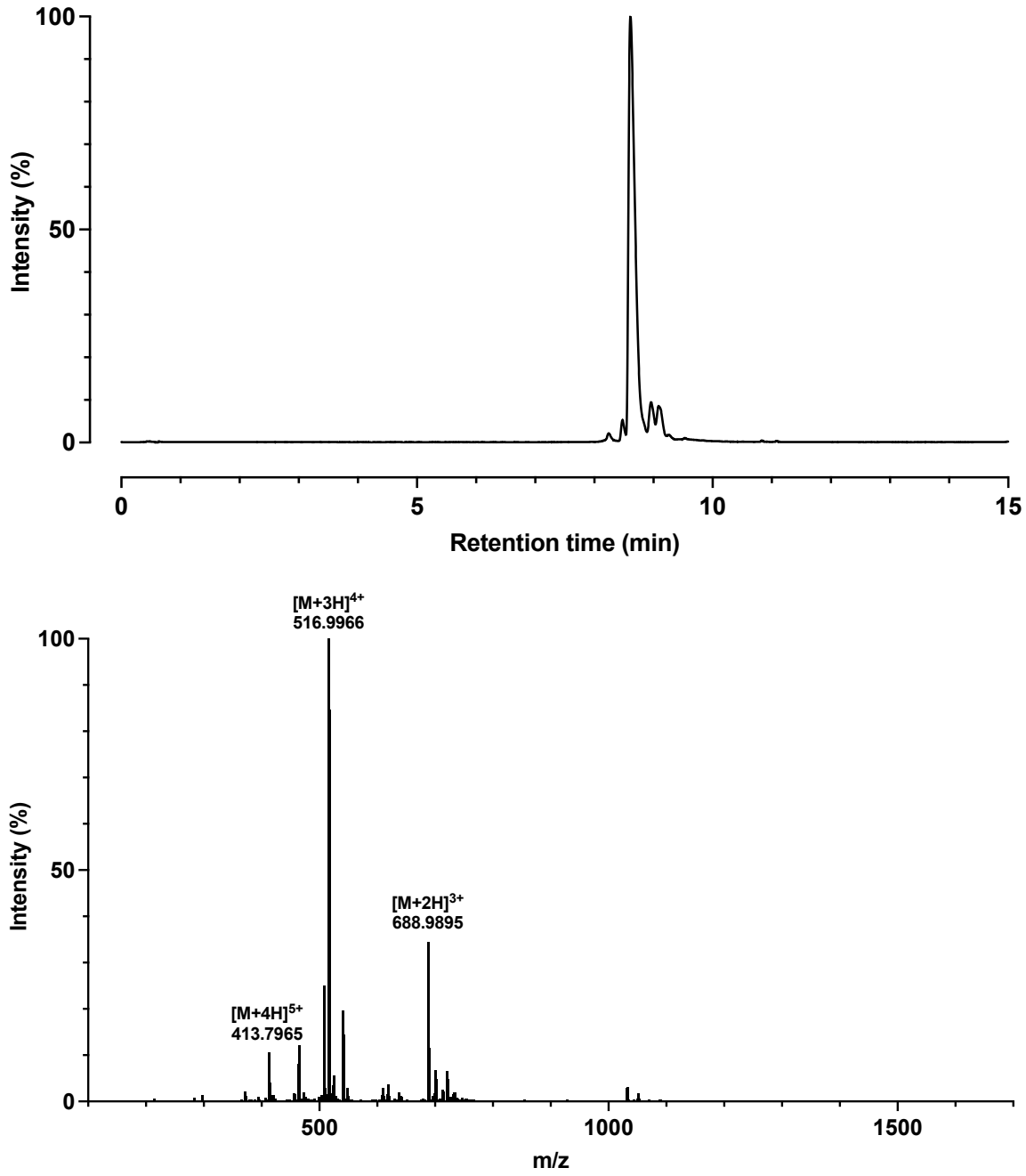


Figure S14. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-1-RhB** and its MS spectrum.

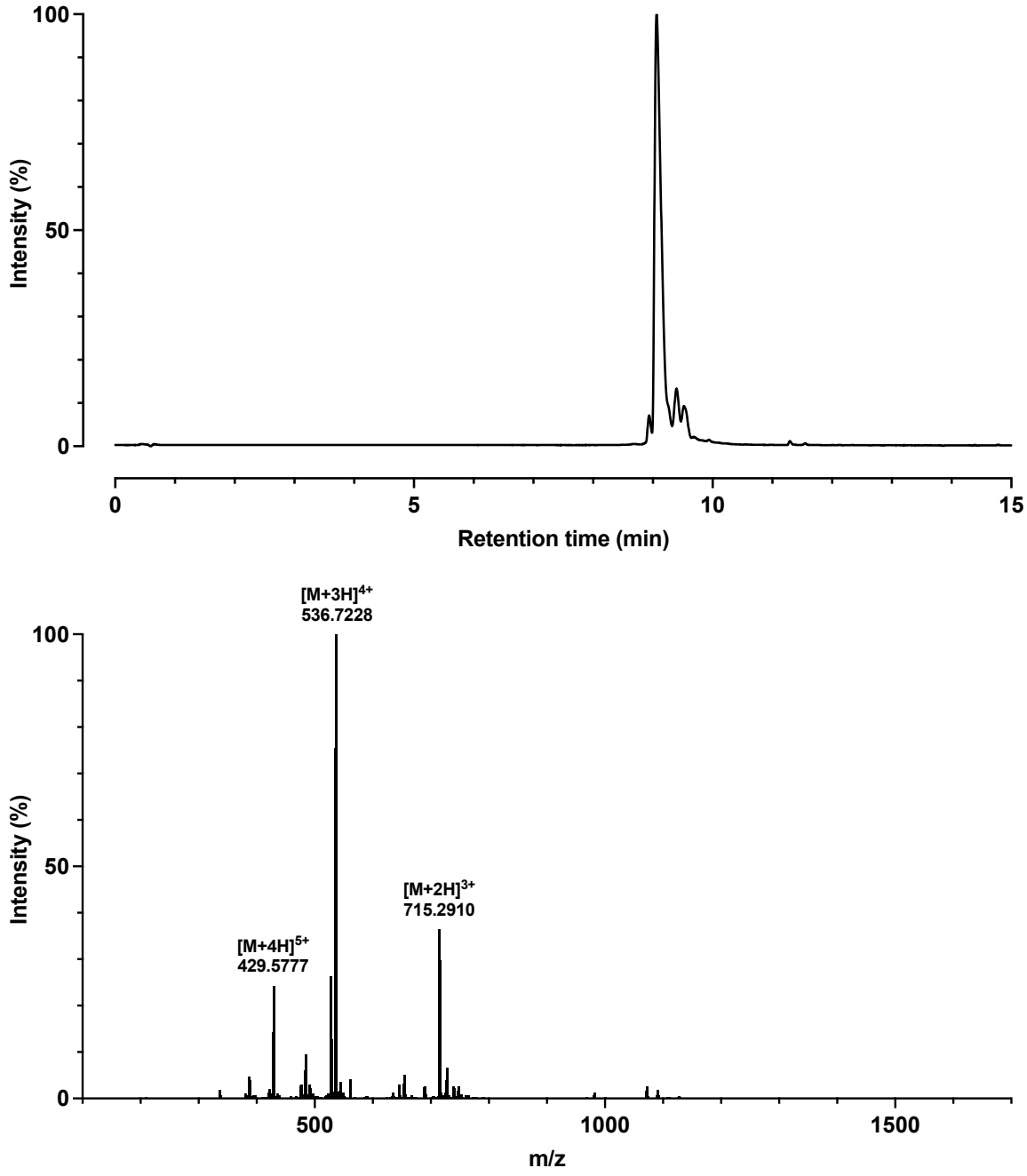


Figure S15. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-1-RhBBr** and its MS spectrum.

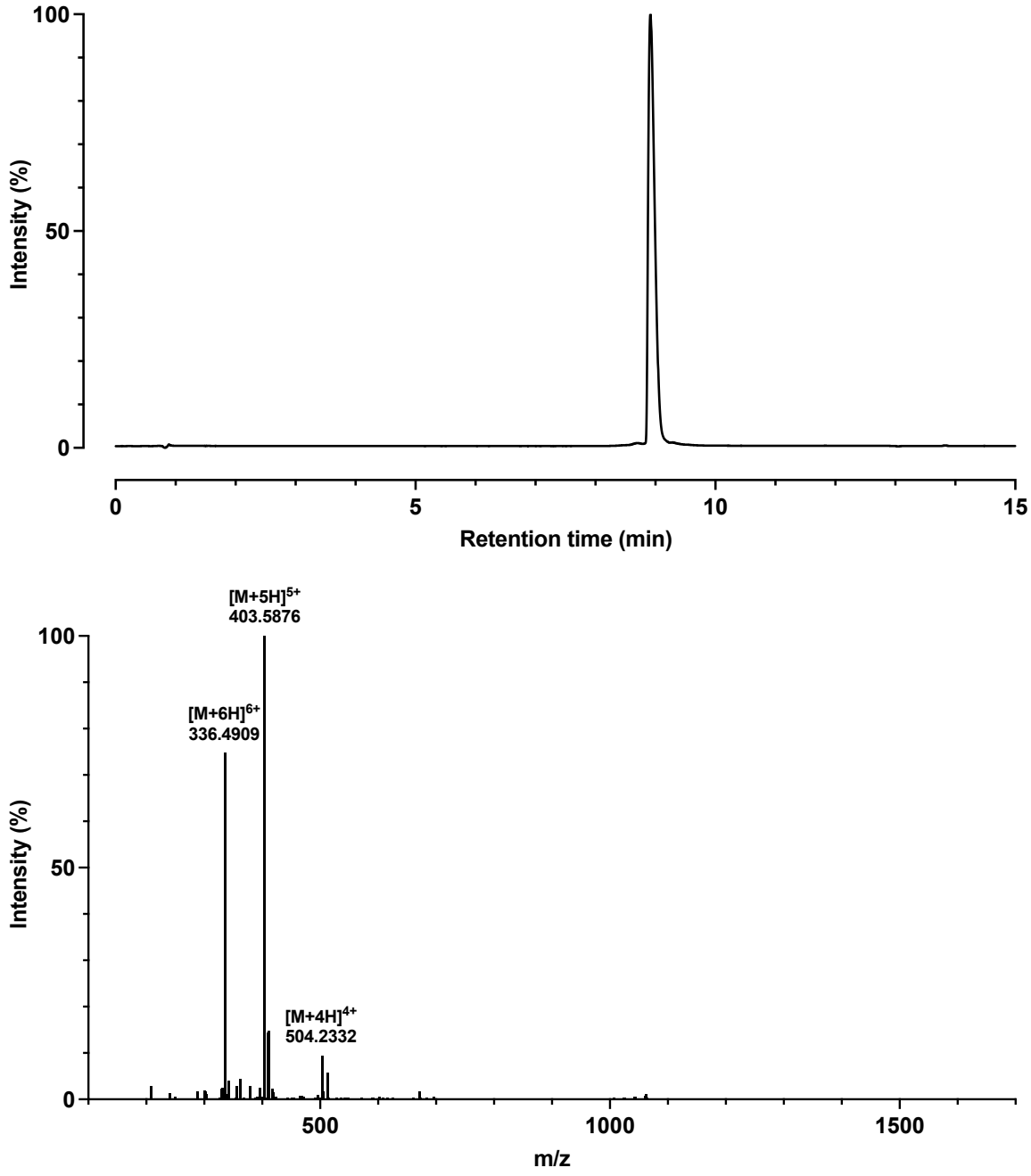


Figure S16. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-2** and its MS spectrum.

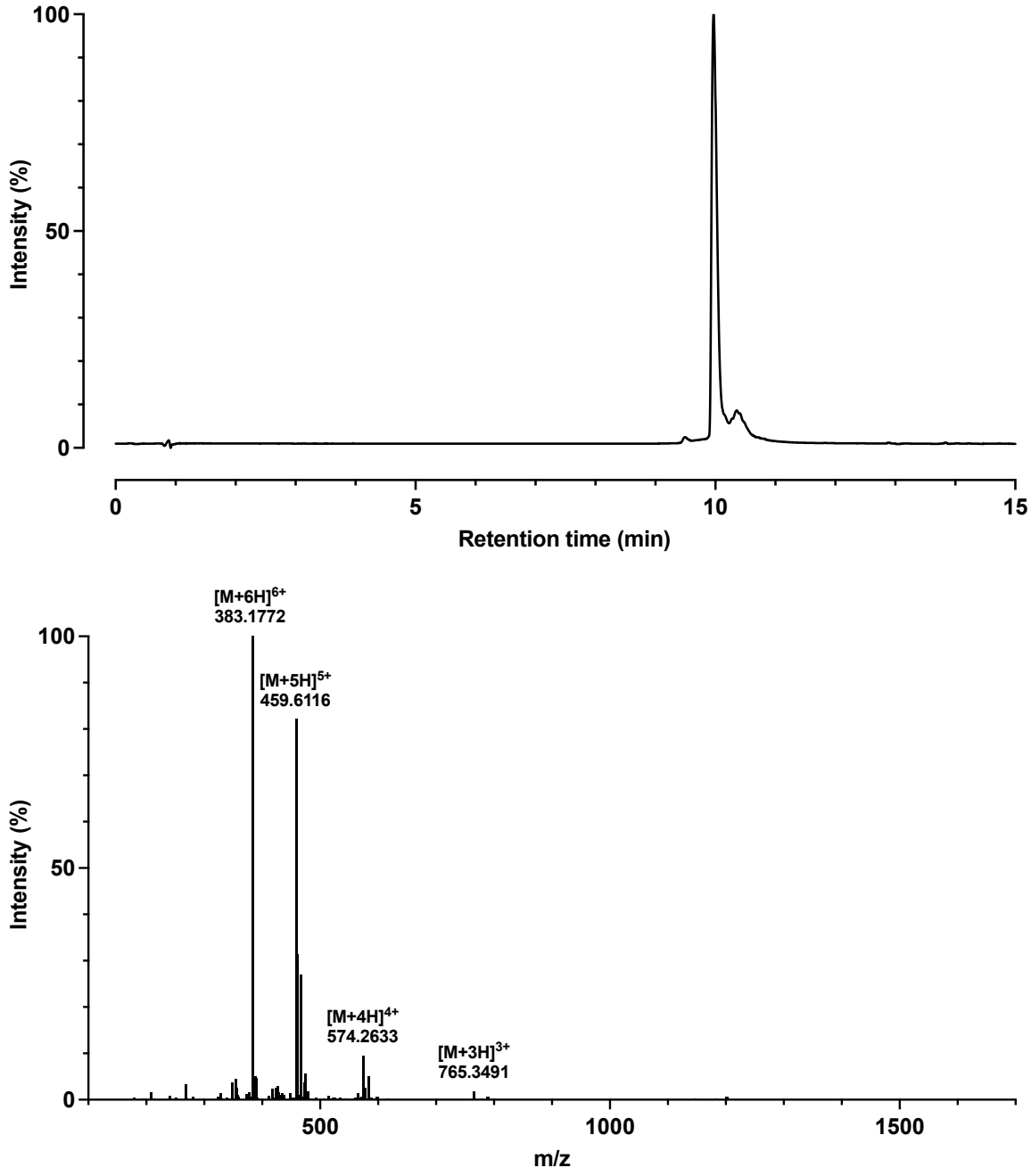


Figure S17. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-2-Np** and its MS spectrum.

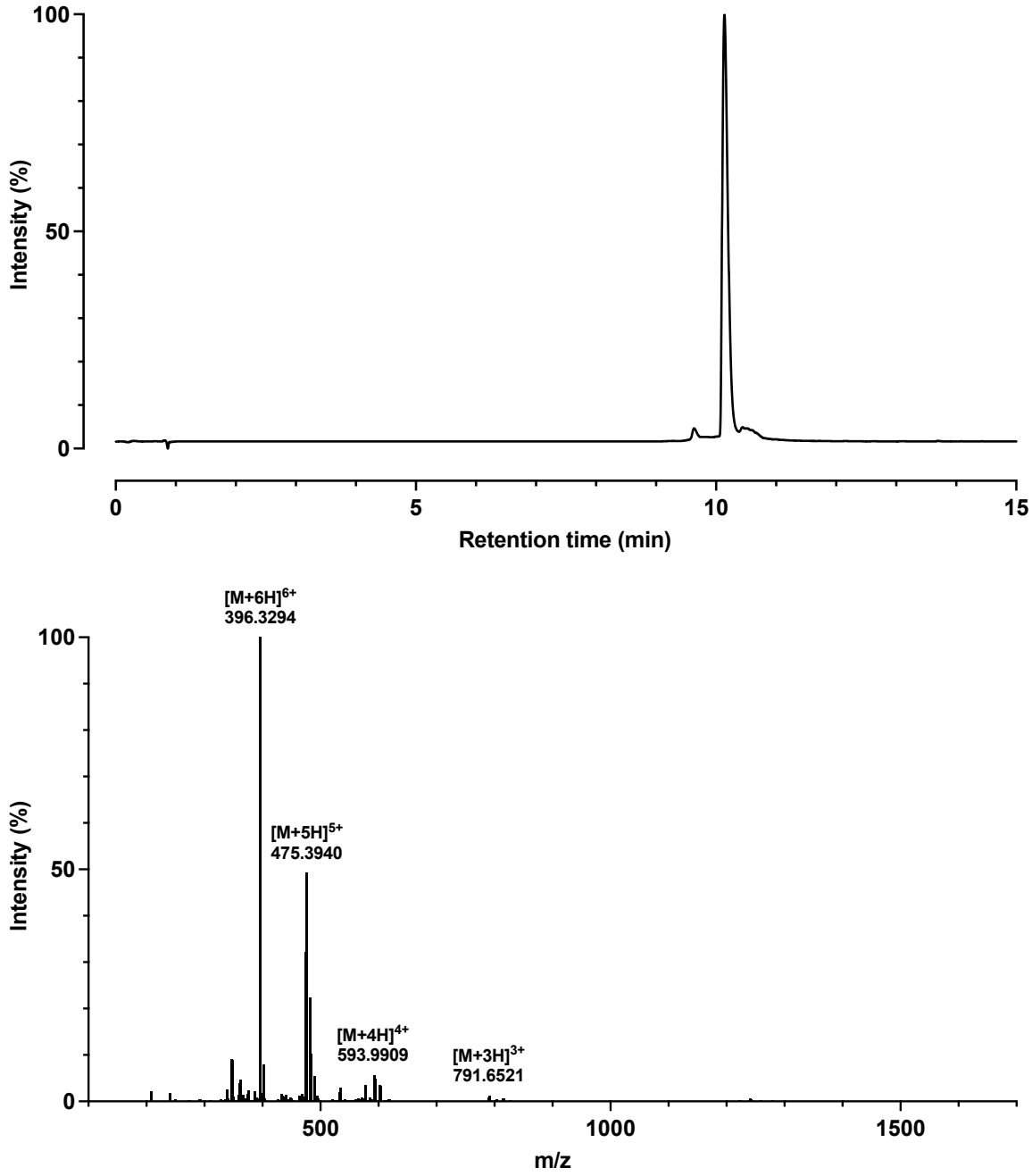


Figure S18. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-2-NpBr** and its MS spectrum.

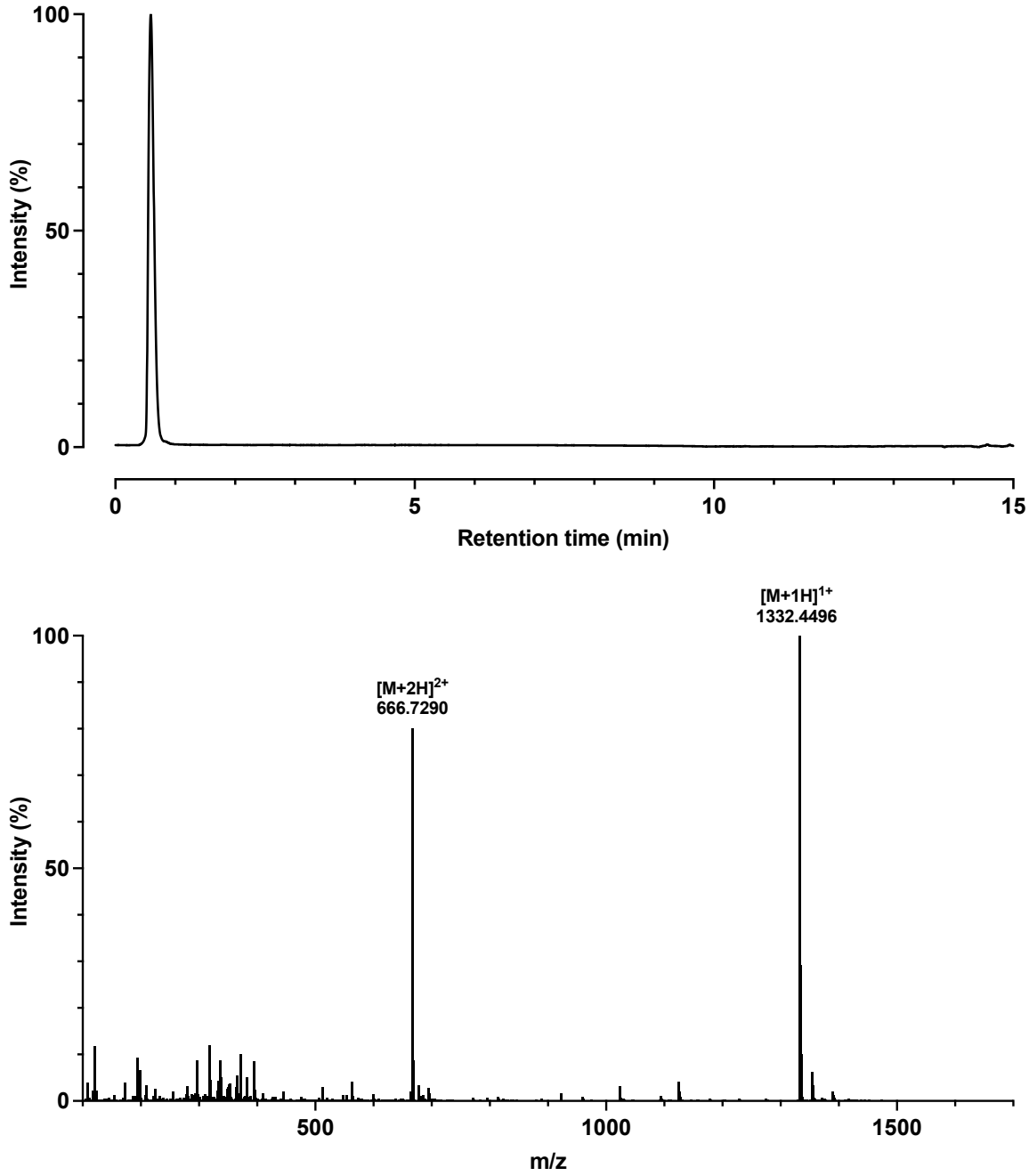


Figure S19. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-3** and its MS spectrum.

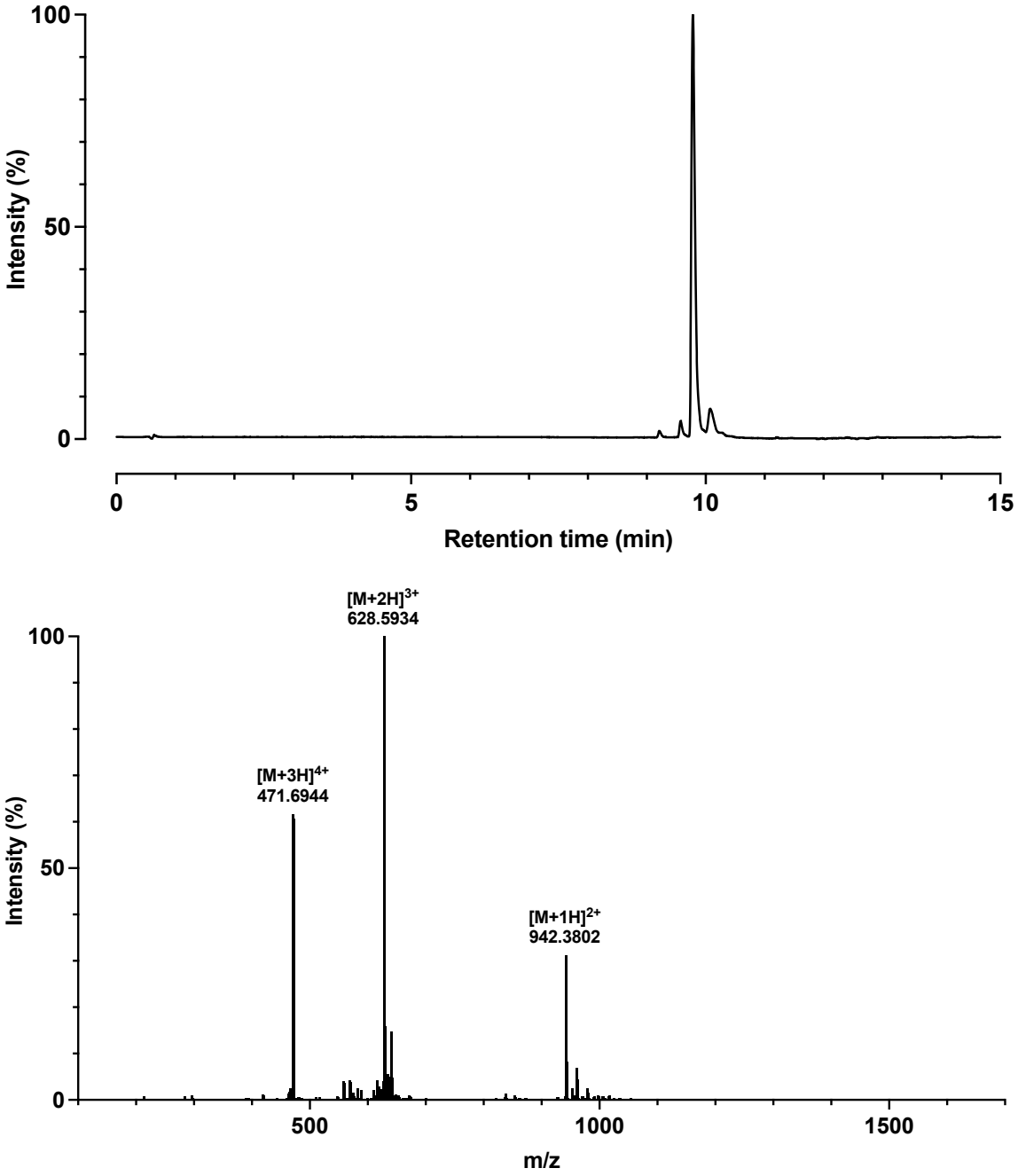


Figure S20. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-3-RhB** and its MS spectrum.

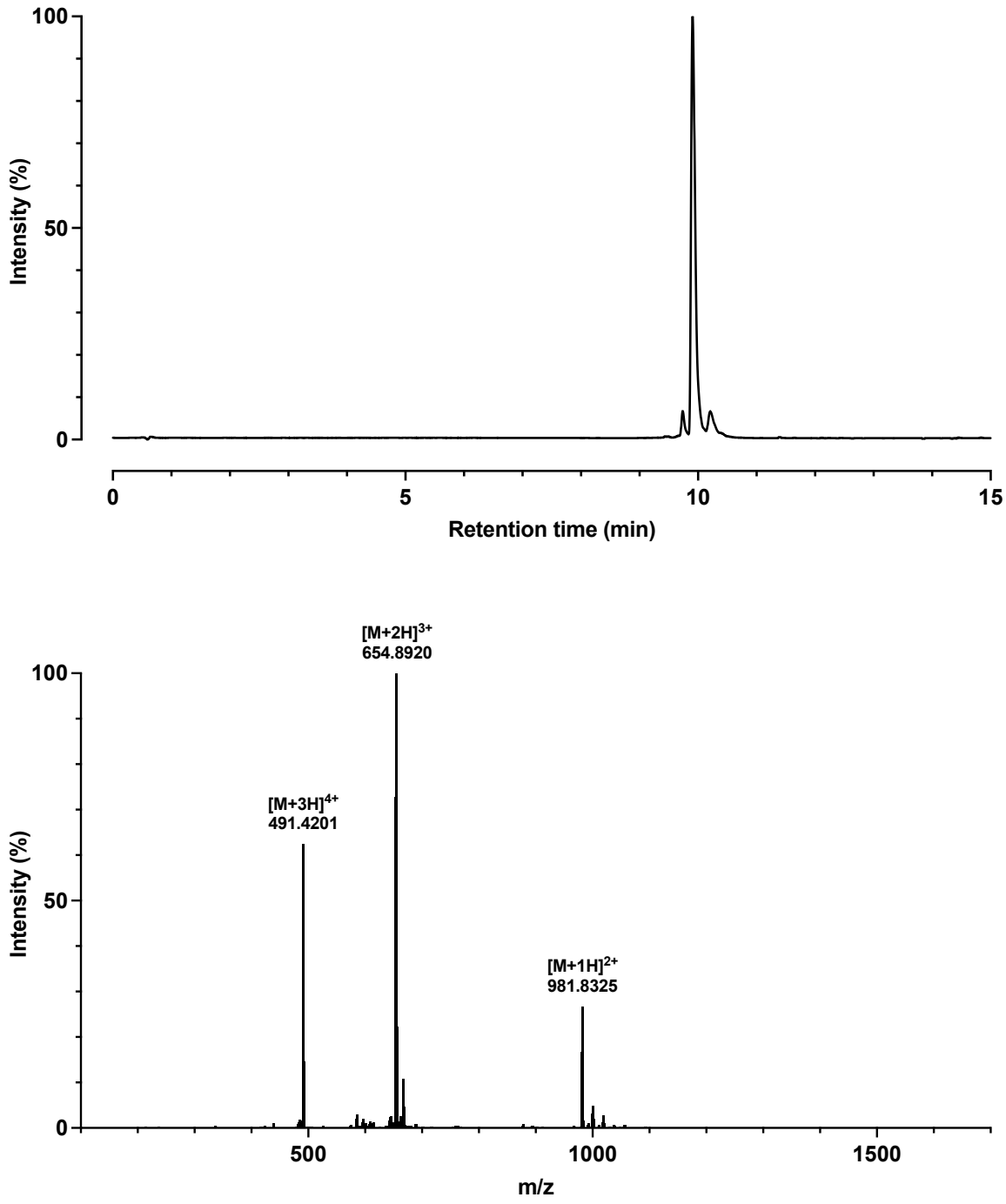


Figure S21. LC-MS chromatogram (254 nm, method A) of purified **Bi-CPP-3-RhBBr** and its MS spectrum.

Supporting X-ray fluorescence data

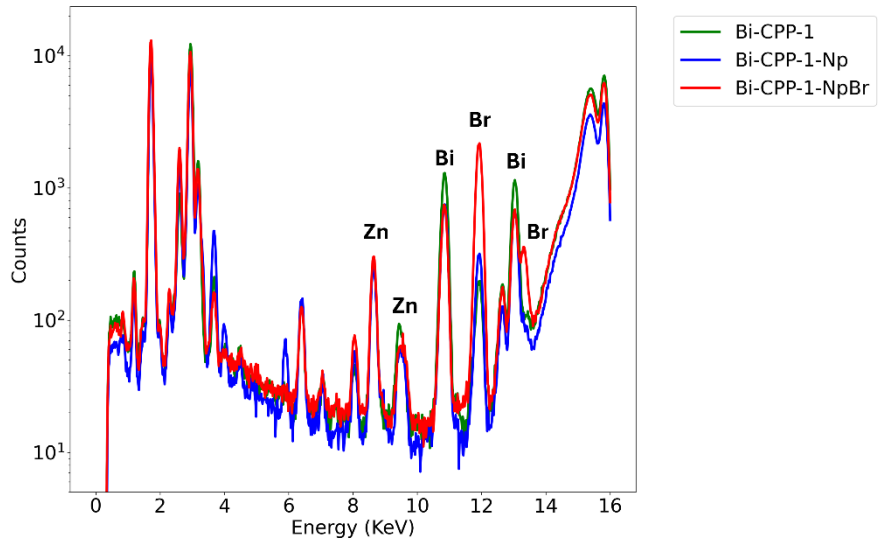


Figure S22. X-ray fluorescence spectra integrated across SKOV-3 cells (*n* = 25 cells for **Bi-CPP-1**, 10 cells for **Bi-CPP-1-Np**, 28 cells for **Bi-CPP-1-NpBr**) treated for 4 h with 10 µM Np derivatives of Bi-CPP-1. Quantitative fits of these per cell spectra generate the respective data points in Figure 7.

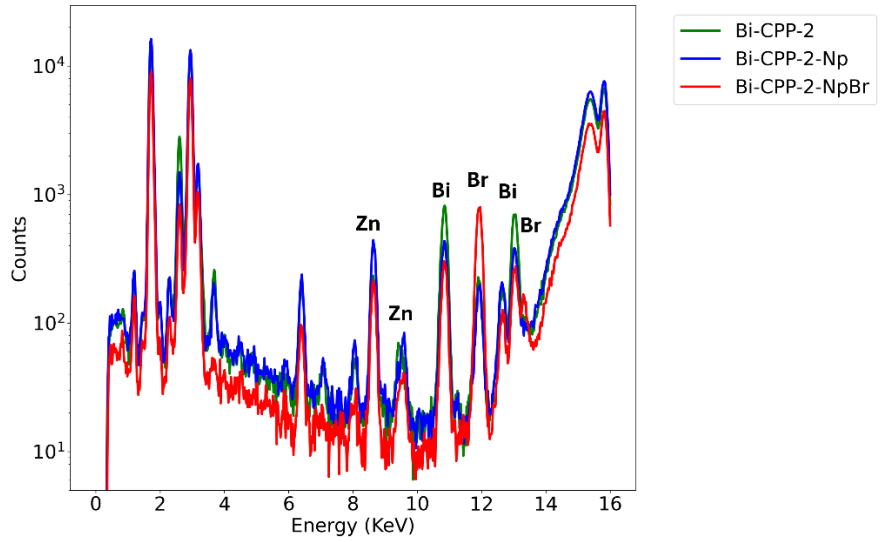


Figure S23. X-ray fluorescence spectra integrated across SKOV-3 cells (*n* = 40 cells for **Bi-CPP-2**, 26 cells for **Bi-CPP-2-Np**, 59 cells for **Bi-CPP-2-NpBr**) treated for 4 h with 10 µM Np derivatives of **Bi-CPP-2**. Quantitative fits of these per cell spectra generate the respective data points in Figure 7.

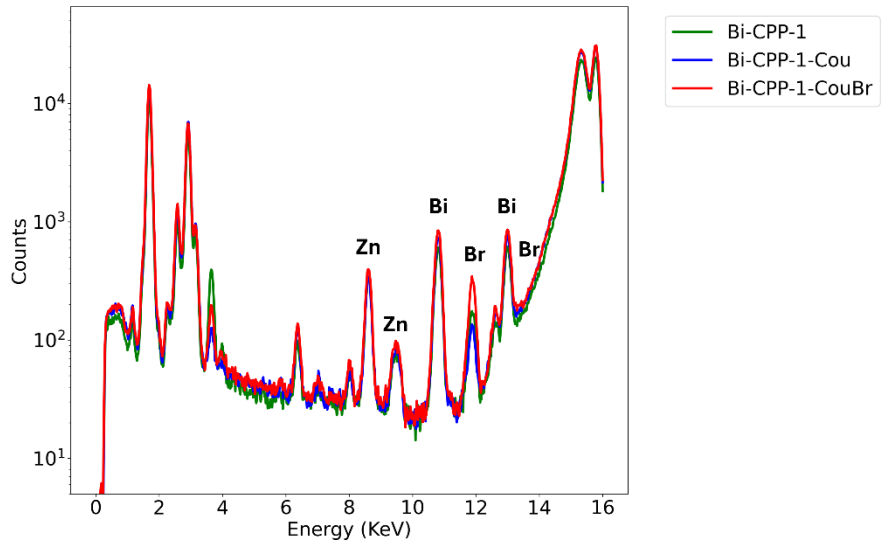


Figure S24. X-ray fluorescence spectra integrated across SKOV-3 cells (n = 25 cells for **Bi-CPP-1**, 27 cells for **Bi-CPP-1-Cou**, 40 cells for **Bi-CPP-1-CouBr**) treated for 4 h with 10 μM Cou derivatives of **Bi-CPP-1**. Quantitative fits of these per cell spectra generate the data points in Figure 7.

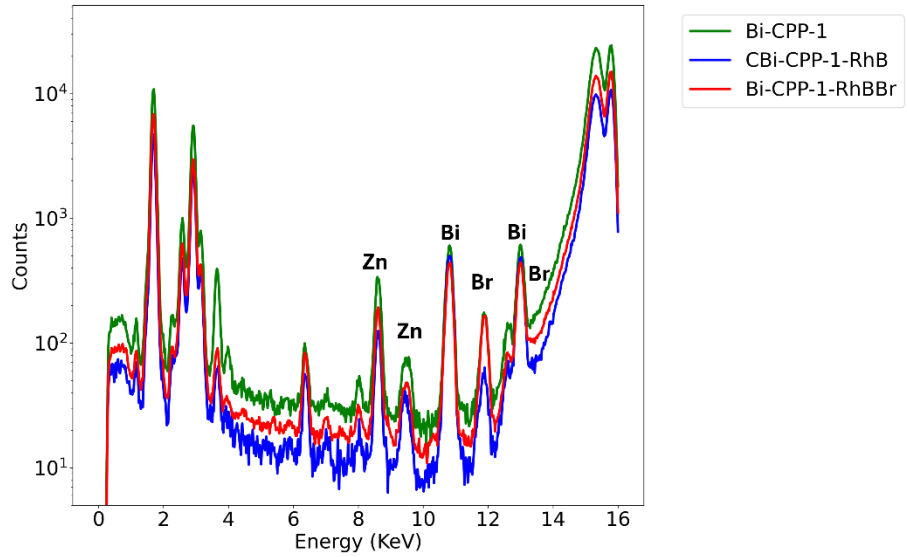


Figure S25. X-ray fluorescence spectra integrated across SKOV-3 cells (n = 28 cells for **Bi-CPP-1**, 50 cells for **Bi-CPP-1-RhB**, 39 cells for **Bi-CPP-1-RhBBr**) treated for 4 h with 10 μM RhB derivatives of **Bi-CPP-1**. Quantitative fits of these per cell spectra generate the data points in Figure 7.

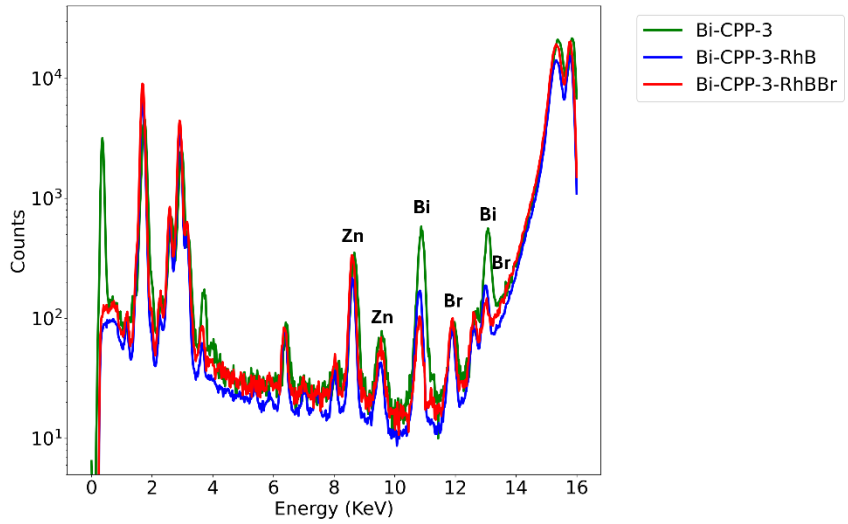


Figure S26. X-ray fluorescence spectra integrated across SKOV-3 cells (n = 14 cells for **Bi-CPP-1**, 27 cells for **Bi-CPP-3-RhB**, 35 cells for **Bi-CPP-3-RhBBr**) treated for 4 h with 10 μM RhB derivatives of **Bi-CPP-1**. Quantitative fits of these per cell spectra generate the data points in Figure 7.

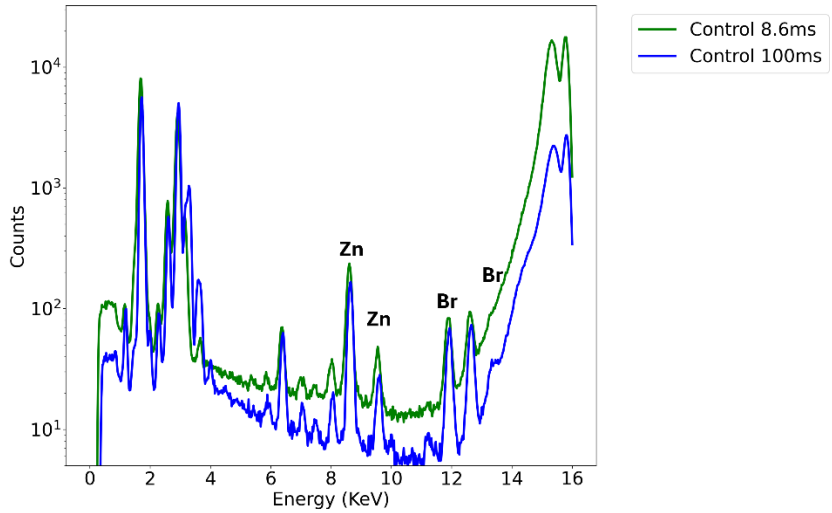


Figure S27. X-ray fluorescence spectra of SKOV-3 **control** cells incubated for 4 h at 8.6 ms (n = 22 cells) and 100 ms (n = 14 cells) dwell. Quantitative fits of these per cell spectra generate the data points in Figure 7.

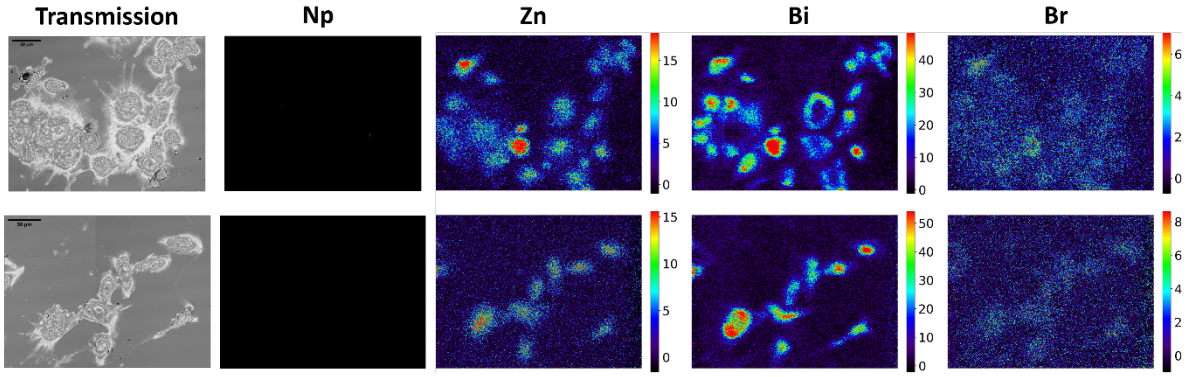


Figure S28. SKOV-3 cells treated with **Bi-CPP-1**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

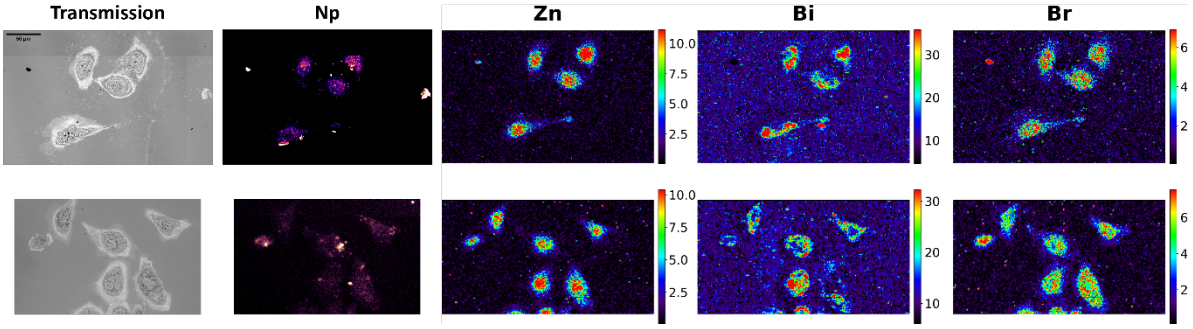


Figure S29. SKOV-3 cells treated with Np-appended **Bi-CPP-1-Np**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

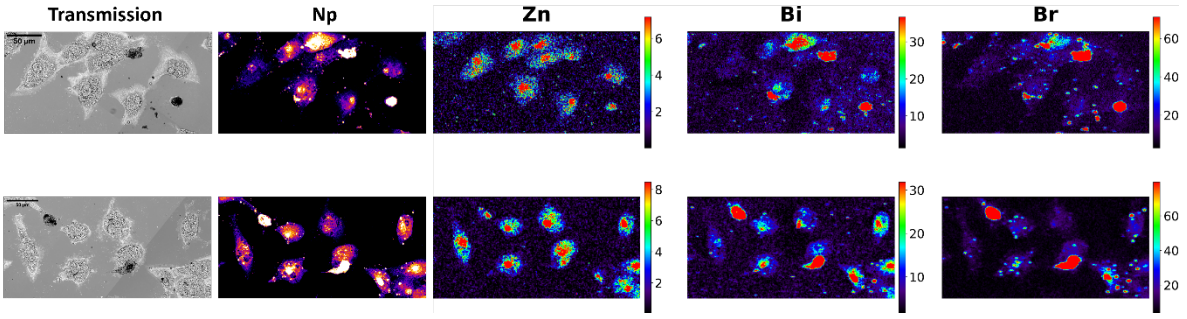


Figure S30. SKOV-3 cells treated with brominated-Np-appended **Bi-CPP-1-NpBr**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

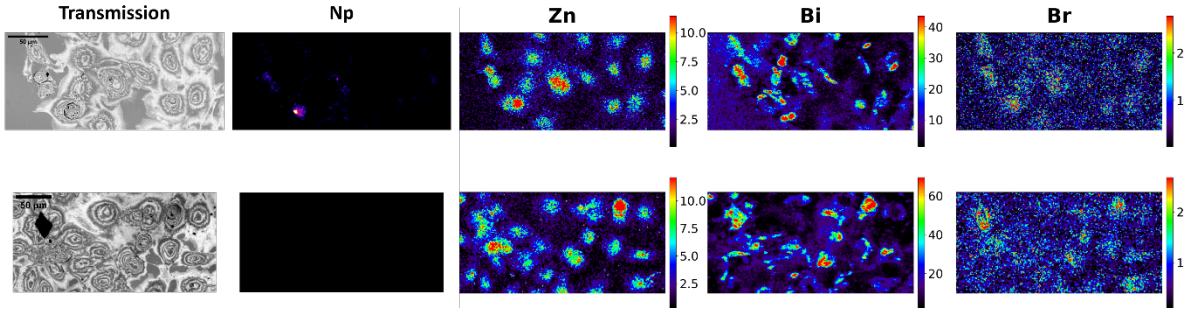


Figure S31. SKOV-3 cells treated with **Bi-CPP-2**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

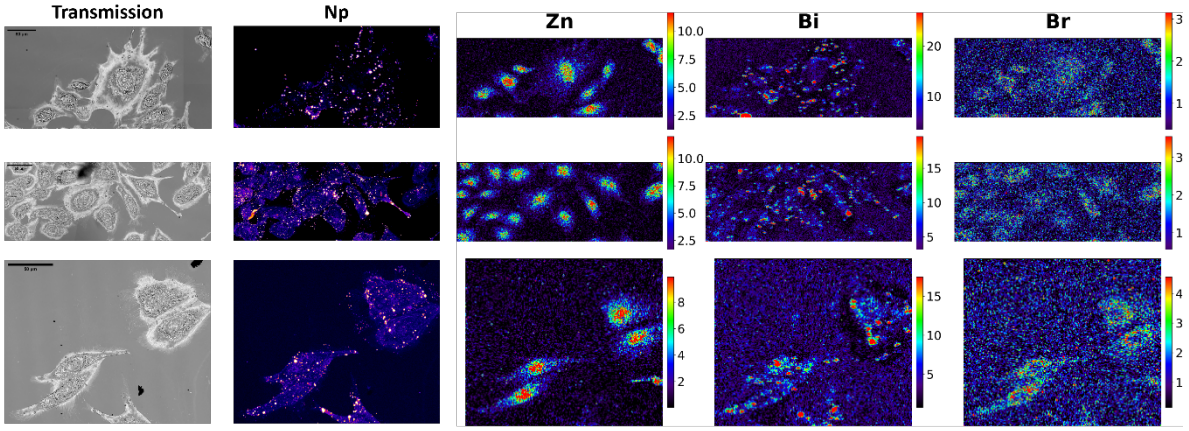


Figure S32. SKOV-3 cells treated with Np-appended **Bi-CPP-2-Np**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

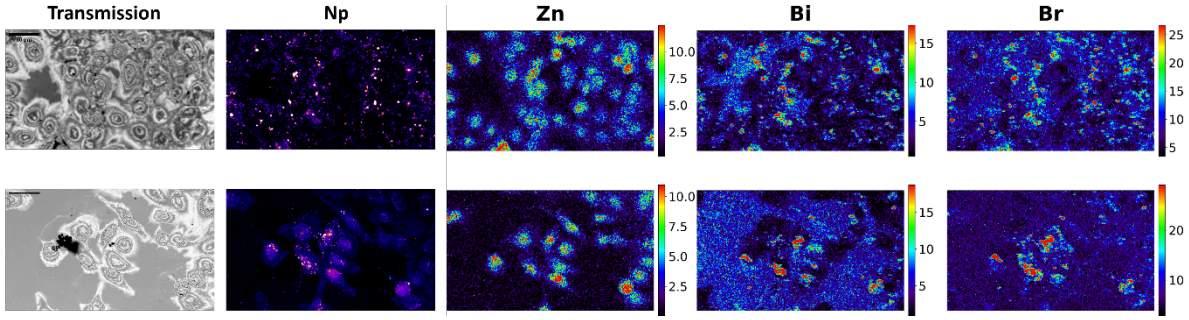


Figure S33. SKOV-3 cells treated with brominated-Np-appended **Bi-CPP-2-NpBr**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

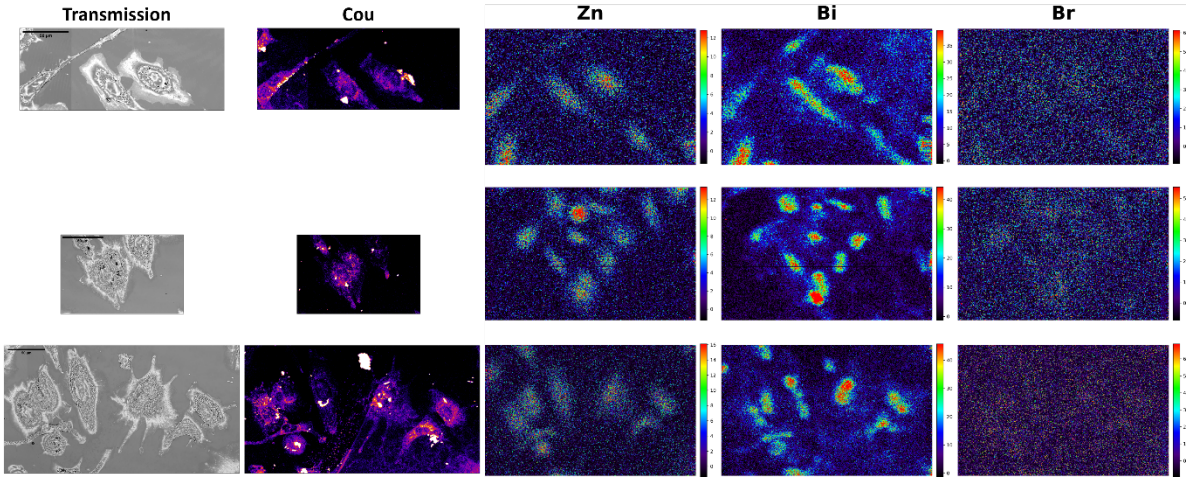


Figure S34. SKOV-3 cells treated with Cou-appended **Bi-CPP-1-Cou**. Optical transmission, fluorescence (Cou) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

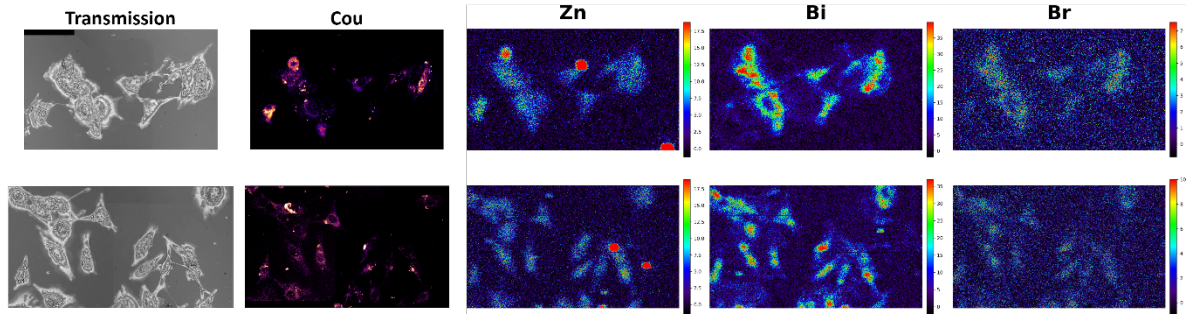


Figure S35. SKOV-3 cells treated with brominated-Cou-appended **Bi-CPP-1-CouBr**. Optical transmission, fluorescence (Cou) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

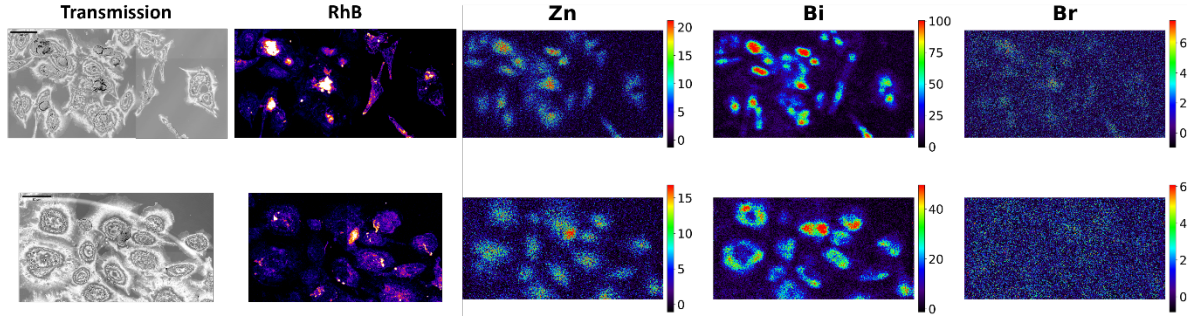


Figure S36. SKOV-3 cells treated with RhB-appended **Bi-CPP-1-RhB**. Optical transmission, fluorescence (RhB) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

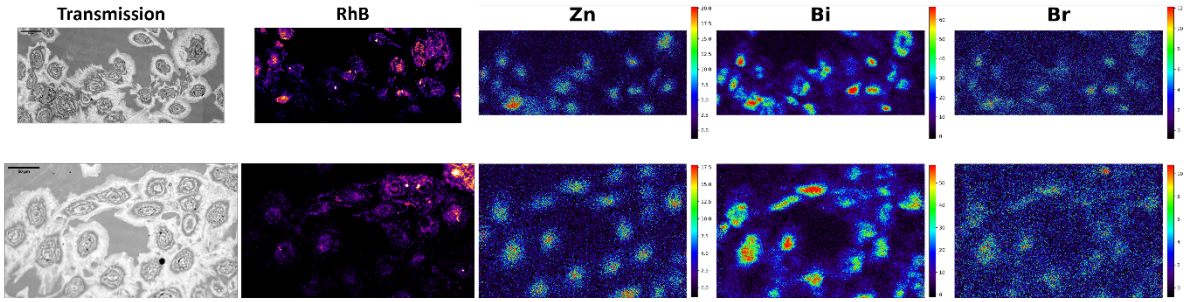


Figure S37. SKOV-3 cells treated with brominated-RhB-appended **Bi-CPP-1-RhBBr**. Optical transmission, fluorescence (RhB) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

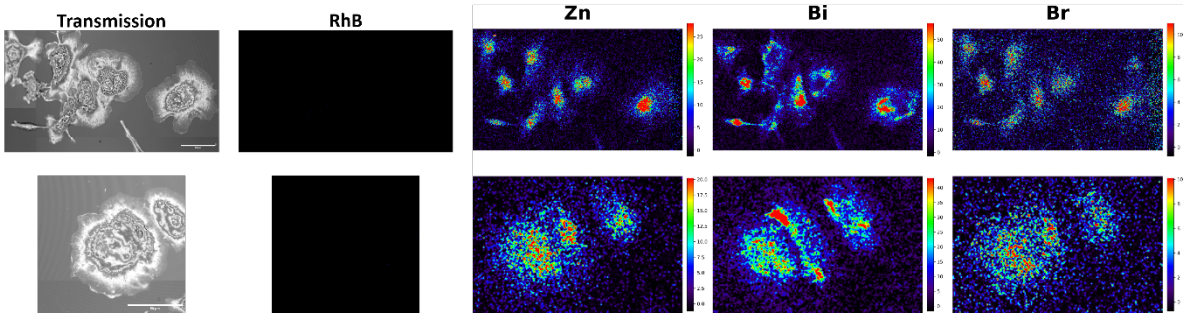


Figure S38. SKOV-3 cells treated with **Bi-CPP-3**. Optical transmission, fluorescence (RhB) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

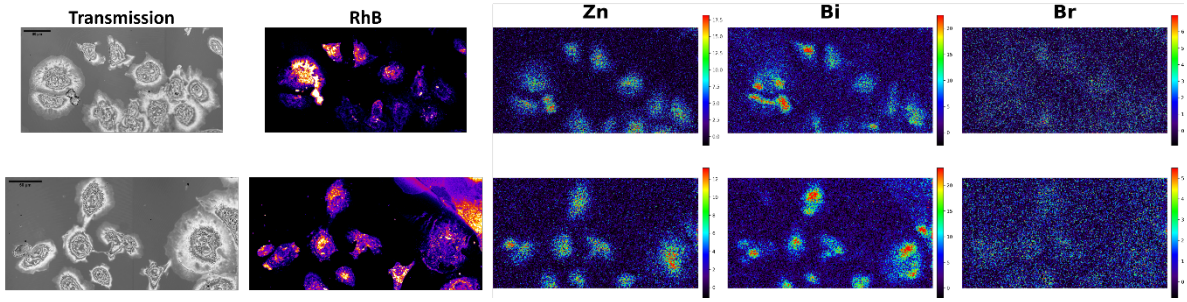


Figure S39. SKOV-3 cells treated with RhB-appended **Bi-CPP-3-RhB**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

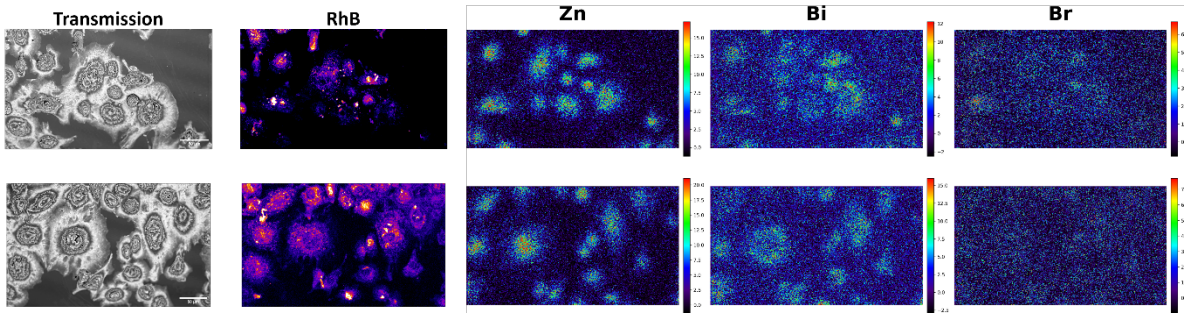


Figure S40. SKOV-3 cells treated with brominated-RhB-appended **Bi-CPP-3-RhBBr**. Optical transmission, fluorescence (Np) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

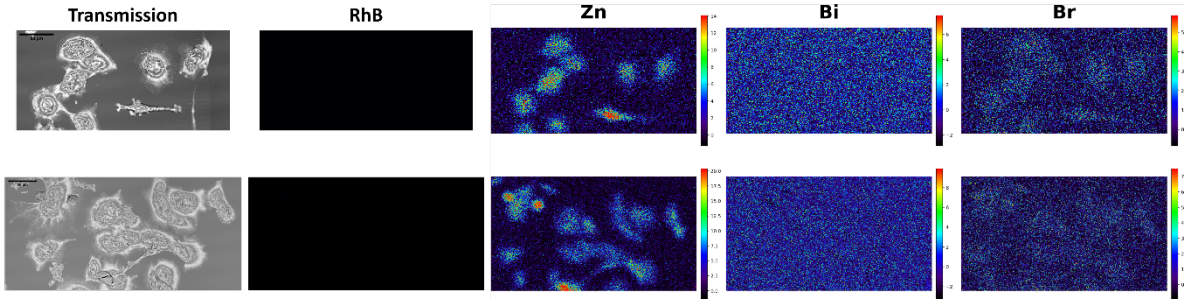
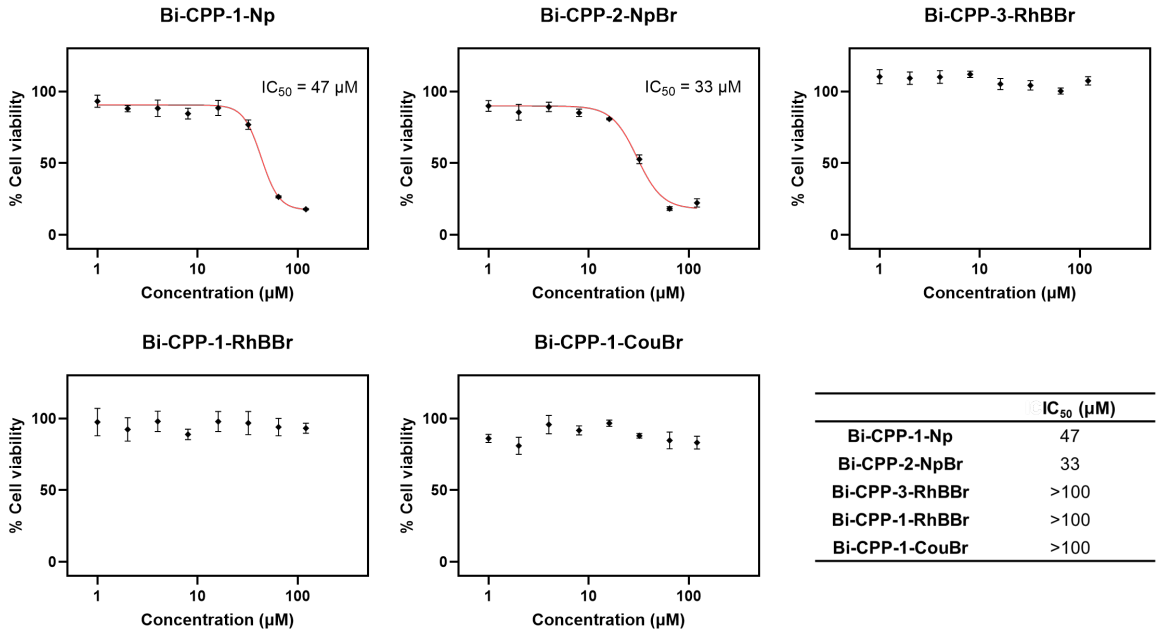


Figure S41. SKOV-3 **control** cells**.** Optical transmission, fluorescence (RhB) and Bi, Br and Zn (ng/cm^2) images. Scale bar is 50 µm.

MTT assay cytotoxicity data



	IC_{50} (µM)
Bi-CPP-1-Np	47
Bi-CPP-2-NpBr	33
Bi-CPP-3-RhBBr	>100
Bi-CPP-1-RhBBr	>100
Bi-CPP-1-CouBr	>100

Figure S42. Dose response-curves from MTT assays (SKOV-3 cells, 24 h incubation) with half maximal inhibitory concentration indicated.

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