Fluorine-18 Incorporation and Radiometal Coordination of Macropa Chelators for PET Imaging and Targeted Alpha Therapy

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List of Acronyms

DCM	dichloromethane
DMSO	dimethyl sulfoxide
EDTA	ethylenediaminetetraacetic acid
EtOAc	ethyl acetate
HPLC	high-performance liquid chromatography
HR-DART-MS	high-resolution direct analysis in real time mass spectrometry
HR-ESI-MS	high-resolution electrospray ionization mass spectrometry
iTLC-SG	instant thin layer chromatography impregnated with a silica gel
K_{222}	Kryptofix® 222
MeOH	methanol
MES	2-(*N*-morpholino)ethanesulfonic acid
PBS	phosphate-buffered-saline
PTFE	polytetrafluoroethylene
RCC	radiochemical conversion
RCY	radiochemical yield
THF	tetrahydrofuran
TLC	analytical thin layer chromatography
UV-vis	ultraviolet-visible

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1. General Remarks

Unless otherwise stated reactions requiring exclusion of oxygen and moisture were carried out in heat-gun dried flasks under nitrogen atmosphere using Schlenk-technique.
All chemicals and solvents were purchased commercially and were used without further purification. The organic solvents used for reactions were of ACS grade or higher. Solvents marked as dry were obtained following storage over preactivated 3 Å molecular sieves. Deuterated solvents were used from Cambridge Isotope Laboratories. Deionized water (≥18 MΩ·cm) was obtained from an Elga Purelab Flex 2 water purification system.

NMR measurements were performed on a 400 MHz Bruker AVIII HD with a BBFO probe, 500 MHz Bruker AVIII HD with a BBO Prodigy cryoprobe, or a 500 MHz Bruker AVIII with a BBO Prodigy cryoprobe. Chemical shifts are reported in parts per million (ppm) in δ units relative to chloroform-*d* (δ_H = 7.26 ppm; δ_C = 77.2 ppm), methanol-d_4 (δ_H = 3.31 ppm; δ_C = 49.0 ppm) or in D_2O which was spiked with MeCN as an internal reference (δ_H = 2.06 ppm, δ_C = 1.47 ppm).[1] Analyses followed first order and the following abbreviations were used throughout: s = singlet, d = doublet, t = triplet, dd = doublet of doublet etc., m = multiplet. Coupling constants (*J*) are given in Hz and refer to H, H-couplings.

HR-MS were recorded on an Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA). As ionization mode were either used ESI or DART, both in the positive ion-detection mode. For ESI, samples were dissolved in HPLC-grade solvents such as acetonitrile, methanol or deionized water. DART samples were directly dosed into the DART ion source. The molecular ions are reported as mass to charge (*m/z*) relation.

Elemental analyses (C,H,N,F) of organic compounds were measured by Atlantic Microlab Inc. (Norcross, GA). The compounds were analysed by combustion using automatic analysers and flask combustion followed by ion chromatography. The results are given in weight percentages.

TLC was carried out on aluminium TLC plates purchased by Merck, silica gel coated with fluorescent indicator F254 (40x 80mm, SIL G/UV254, 0.2 mm layer thickness). Detection was carried out using UV-light (254 nm or 366 nm).

Flash column chromatography was carried out using a Biotage Isolera™ One Flash Chromatography system with cartridges (Sfär chromatography columns) filled with silica gel purchased from SiliCycle™ SiliaFlash™ (irregular silica gel, F60, 40–63 µm, 60 Å) or C18-functionalized silica gel cartridge (Sfär C18 duo reversed phase). For normal-phase purification, organic mobile phase mixtures of different solvents (hexanes/EtOAc or DCM/methanol) were used and for reverse-phase purification, solvent mixtures of methanol and deionized water with 0.1% v/v trifluoroacetic acid (TFA) as additive were used.

HPLC systems were used for preparative purification at room temperature: LC-20AP preparative system (Shimadzu, Japan), equipped with a CBM-20A communications bus module, and an SPD-20AV UV-vis detector for wavelength detection at 254 nm. The data was processed with LabSolutions CS software. The preparative HPLC was performed on a C_{18}-functionalized Epic Polar preparative column (ES Industries, West Berlin, NJ, 120 Å, 10 µm, LC Column 25 cm×20 mm). The purification was performed using the following method: 10% MeOH/90% H_2O+0.1% trifluoroacetic acid (TFA) (0–5 min), 10–100% MeOH+0.1% TFA (5–35 min) and a flow rate of 14 mL/min (*system 1*).

Analytical HPLC measurements were performed at room temperature using the analytical LC-20AT pump with the CBM-20A communication module equipped with the DGU-20A degassing unit (Shimadzu, Japan). The HPLC runs were performed on a C_{18}-functionalized column (Restek, Bellefonte, PA, USA, 100 Å, 5 µm, LC Column 25 cm×4.6 mm). In addition, the analytical measurements were performed using the following method: 10% MeOH/90% H_2O+0.1% TFA (0–5 min), 10–100% MeOH+0.1% TFA (5–25 min) and a flow rate of 1 mL/min (*system 2*). For radio-HPLC measurements, the instrument was coupled to the Flow-Ram system and the Laura software (Lablogic Systems Ltd, Sheffield, UK). The radioactivity was detected via a sodium iodide radio detector. The radio-HPLC for the [^{203}Pb][Pb(macropa-F)] complex was performed on the C_{18}-functionalized Luna® column (Phenomenex, Torrance, CA, USA, 100 Å, 5 µm, LC Column

15 cm×3 mm). The following method was used: 0% MeOH/100% H_2O+0.1% TFA (0–5 min), 0–90% MeOH+0.1% TFA (5–25 min) and a flow rate of 0.5 mL/min (*system 3*).

The purification of the radiofluorinated compound was performed by semi-preparative HPLC on a Sykam S1122 Solvent Delivery System by using a XBridge C18-functionalized preparative column (Waters Corporation, Milford, MA, 130 Å, 5 µm, LC Column 25 cm×10 mm) and a wavelength UV detector set at 220 nm. The purification was performed by using an isocratic mobile phase of deionized water containing acetic acid solution (83%) and methanol (17%) with a flow rate of 5 mL/min (*system 4*). Additionally, the $[^{18}F][Bi(macropa\text{-}F)]^+$ and $[^{18}F][Pb(macropa\text{-}F)]$ complexes were purified using a Sep-Pak C18 Plus Light cartridge (130 mg sorbent, particle size 55–105 µm).

The fluorine-18 radiolabeled compounds and crude mixtures were analyzed using a Thermo Scientific Dionex Ultimate 3000 UHPLC system equipped with a diode array detector and a radiation detector (Model 105S, Carrol & Ramsey Instruments) at room temperature. The measurements were carried out on an analytical C18-functionalized XBridge column (Waters Corporation, Milford, MA, 130 Å, 5 µm, LC Column 15 cm × 4.6 mm) monitoring the wavelength at 254 nm. The following gradient method was used: 10% MeOH/90% ammonium acetate (10 mM, pH = 4.5) (0 min), 10–100% MeOH (0–10 min), 100% MeOH (10–10.5 min), 100–10% MeOH (10.5–15 min) and a flow rate of 1 mL/min (*system 5*). The identity of the radiofluorinated compounds were confirmed by the comparison with the corresponding non-radioactive reference standard.

Chemical structures were drawn with ChemDraw Professional 22.2.0.3300.

NMR data were analyzed with MestReNova 14.3.0-30573.

Radiolabelling with fluorine-18, lead-203, and bismuth-207. The $[^{18}F]KF$ was produced via a $^{18}O(p, n)^{18}F$ reaction by bombardment of enriched $[^{18}O]$water with 16 MeV protons using a PETtrace cyclotron from GE Healthcare Technologies, Inc. (Chicago, IL, USA) at Massachusetts General Hospital. The cyclotron was operated at 65 µA for 6 min, producing approximately 500 mCi (18.5 GBq) of $[^{18}F]$fluoride.

$[^{203}Pb]PbCl_2$ in diluted hydrochloric acid (1 N) was produced via a $^{205}Tl(p,3n)^{203}Pb$ reaction and was purchased from the U.S. Department of Energy Isotope Program and University of Alabama at Birmingham, AL, USA.

[^{207}Bi]Bi$(NO_3)_3$ in diluted nitric acid (4 N) was purchased from Los Alamos National Laboratory, NM, USA and its Isotope Production and Application Program stock supply. The purity of [^{207}Bi]Bi$(NO_3)_3$ was verified by ICP-OES as >99%.

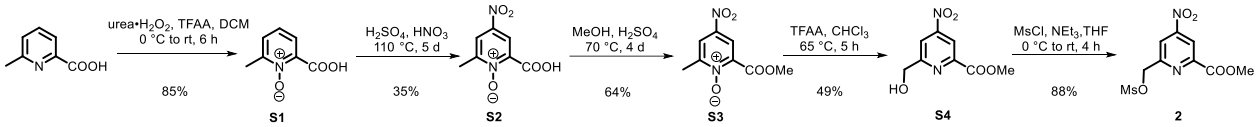
The quantification of [^{203}Pb]Pb^{2+}, [^{207}Bi]Bi^{3+}, and distribution coefficient determination were carried out by using the gamma counter 2470 WIZARD2 Automatic Gamma Counter from Perkin Elmer (Waltham, MA, USA).

Radio-TLC experiments were performed on a Scan-RAM radio-TLC scanner equipped with a sodium iodide detector with a Laura chromatography software v5.0.7 (Lablogic Systems Ltd, Sheffield, UK). Radio-TLC was performed by using glass microfiber chromatography paper impregnated iTLC-SG plates purchased by Agilent (Santa Clara, CA, USA).

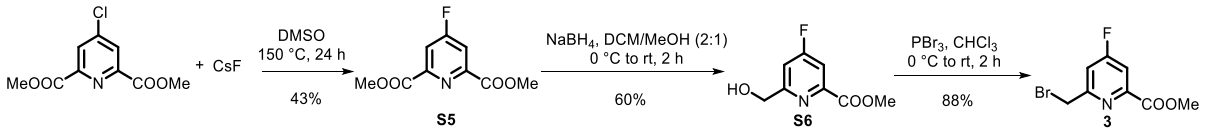
Human serum stability studies were conducted using untreated human blood serum from Sigma Aldrich, (Sera human frozen liquid, #SLCF9666, aseptically filled, S7023–100 mL). The human serum was removed from the freezer and was allowed to thaw to ambient temperature before it was used for the stability studies.

2. Syntheses

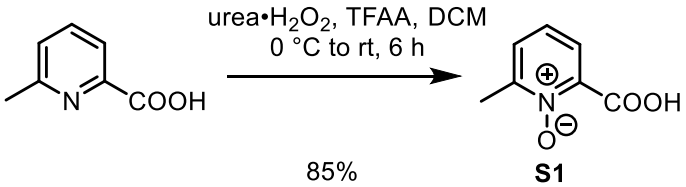
Pendant Arm 1 Synthesis:



Pendant Arm 2 Synthesis:



2.1 Synthesis of *N*-oxide S1



2-Carboxy-6-methyl pyridine (4.00 g, 29.2 mmol, 1 eq.) was dissolved in DCM (200 mL), and the solution was cooled to 0 °C. Then, urea hydrogen peroxide (8.23 g, 87.5 mmol, 3 eq) was added

portion-wise to the colorless solution and was stirred further for 10 min at 0 °C. Trifluoroacetic anhydride (TFAA, 12.2 mL, 87.5 mmol, 3 eq.) was added dropwise to the colorless solution at 0 °C over a time period of 20 min. After another 20 min at 0 °C, the reaction mixture was allowed to warm up to room temperature. The colorless solution was stirred for 4 h at room temperature. After complete conversion of the starting material (monitored by TLC: DCM:MeOH: 95:5), deionized water was added to the solution and the organic phase was separated. The aqueous phase was extracted 3 times with DCM (3×150 mL). The combined organic phases were washed with a saturated solution of sodium chloride (brine). After separation, the organic phase was dried over sodium sulfate. After filtration, the organic solvent was removed under reduced pressure to afford **S1** as a off-white solid (3.80 g, 24.8 mmol, 85%).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K):
δ (ppm) = 8.34 (dd, J = 6.8, 3.1 Hz, 1 H, H_{arom}), 7.62–7.54 (m, 2 H, H_{arom}), 2.66 (s, 3 H, CH_3).

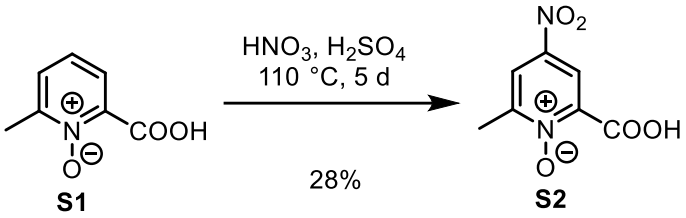
^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$, 298 K):
δ (ppm) = 161.7, 149.4, 137.3, 130.0, 129.9, 127.4, 17.8.

HR-DART-MS (positive, 150 °C):

$C_7H_7NO_3$	calculated *m/z*	found *m/z*
$[M+H]^+$	154.0499	154.0493

The chemical properties are in accordance with the literature.[2]

2.2 Synthesis of *N*-oxide S2



The *N*-oxide **S1** (3.00 g, 19.6 mmol, 1 eq.) was dissolved in concentrated sulfuric acid (96–98%, 21.0 mL) at 0 °C. Then, concentrated nitric acid (70%, 24.0 mL) was added dropwise over 15 min to the slightly yellow solution at 0 °C. The reaction mixture was stirred for 20 min at 0 °C before heating the solution to 110 °C. At 110 °C, the yellow reaction mixture was refluxed for 5 days. During the reaction, a constant release of NO_x gases was observed. After 5 days, the reaction mixture was allowed to cool to room temperature. Then, the acidic solution was carefully added dropwise to ice (800 mL). The obtained beige-colored precipitate was collected via filtration and

was washed with a small amount of deionized water. After drying the sample at 60 °C for 4 h, under high vacuum, **S2** (1.10 g, 5.55 mmol, 28%) was obtained as a pale-green solid.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K):
δ (ppm) = 16.46 (s, 1 H, COOH), 9.07 (d, J = 3.3 Hz, 1 H, H_{arom}), 8.36 (d, J = 3.3 Hz, 1 H, H_{arom}), 2.74 (s, 3 H, CH_3).

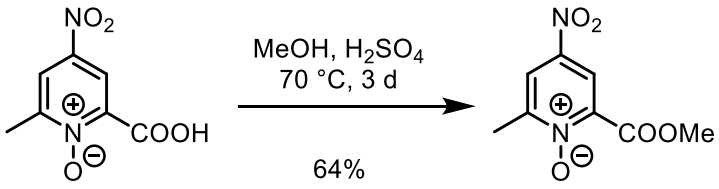
^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, 298 K):
δ (ppm) = 159.5, 152.4, 144.3, 138.7, 123.1, 121.7, 18.4.

HR-ESI-MS (MeCN):

$C_7H_6N_2O_5$	calculated *m/z*	found *m/z*
$[M+H]^+$	199.0350	199.0347

The chemical properties are in accordance with the literature.[2]

2.3 Synthesis of *N*-oxide S3



S2 **S3**

The *N*-oxide **S2** (1.10 g, 5.55 mmol, 1 eq.) was suspended in anhydrous methanol (34.0 mL). Then, concentrated sulfuric acid (96–98%, 250 µL) was added dropwise and under vigorous stirring to the yellow suspension. Afterwards, the reaction mixture was heated to 70 °C and stirred for 3 days. After full consumption of the starting material (monitored by TLC: EtOAc:hexanes: 1:1), the solvent was concentrated under reduced pressure to a final volume of ca. 10 mL. Then, a solution of sodium bicarbonate (5 v/v%, 10 mL) was added to the green solution to neutralize it. After neutralization, DCM (200 mL) was added, and the organic phase was separated. The aqueous phase was extracted 3 times with DCM (3×150 mL). The combined organic phases were washed with brine, and then dried over sodium sulfate. After filtration, the organic phase was evaporated under reduced pressure. The yellow residue was further purified with Biotage flash column chromatography (50 g SiO_2 cartridge, EtOAc:hexanes 10:90 to 100% EtOAc). The product **S3** (750 mg, 3.54 mmol, 64%) was observed as a beige solid.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K):
δ (ppm) = 8.35 (d, J = 3.4 Hz, 1 H, H_{arom}), 8.19 (d, J = 3.4 Hz, 1 H, H_{arom}), 4.04 (s, 3 H, CH_3), 2.58 (s, 3 H, CH_3).

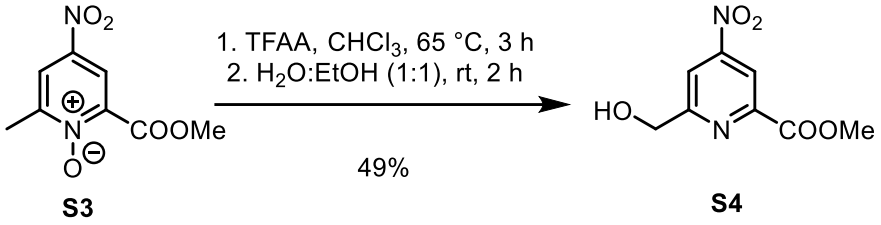
^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, 298 K):
δ (ppm) = 160.8, 152.7, 142.1, 140.5, 121.4, 119.4, 53.9, 18.4.

HR-DART-MS (positive, 150 °C):

$C_8H_8N_2O_5$	calculated *m/z*	found *m/z*
$[M+H]^+$	213.0506	213.0505

The chemical properties are in accordance with the literature.[3]

2.4 Synthesis of Alcohol S4



S3 **S4**

The *N*-oxide **S3** (400 mg, 1.89 mmol, 1 eq.) was dissolved in anhydrous chloroform (35.0 mL). Then, TFAA (5.24 mL, 37.7 mmol, 20 eq.) was added to the yellow solution. The reaction mixture was heated to 65 °C and stirred for 3 h. After full consumption of the starting material (monitored by TLC: EtOAc:hexanes: 1:1), the solvent was removed under reduced pressure to obtain a yellow residue. Then, a 1:1 mixture of deionized water and ethanol (9 mL) was added to the residue. The dark yellow solution was stirred for 2 h at room temperature, after which the ethanol was removed under reduced pressure. Afterwards, a solution of sodium bicarbonate (10 v/v%, 5 mL) was used to neutralize the yellow solution. The aqueous phase was extracted 3 times with DCM (3×250 mL), and the combined organic phases were washed with brine, which were subsequently dried over sodium sulfate. After filtration, the organic phase was evaporated under reduced pressure. The yellow residue was further purified with Biotage flash column chromatography (50 g SiO_2 cartridge, EtOAc:hexanes 10:90 to 100% EtOAc). The product **S4** (195 mg, 919 µmol, 49%) was observed as a pale-yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$, 298 K):
δ (ppm) = 8.69 (d, J = 2.0 Hz, 1 H, H_{arom}), 8.36 (d, J = 2.1 Hz, 1 H, H_{arom}), 5.03 (s, 2 H, CH_2), 4.07 (s, 3 H, CH_3), 2.92 (t, J = 5.4 Hz, 1 H, OH).

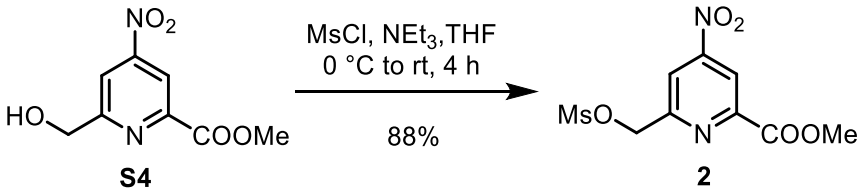
$^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$, 298 K):
δ (ppm) = 164.5, 163.9, 155.6, 150.1, 116.8, 116.5, 64.8, 53.7.

HR-DART-MS (positive, 150 °C):

$C_8H_8N_2O_5$	calculated *m/z*	found *m/z*
$[M+H]^+$	213.0506	213.0511

The chemical properties are in accordance with the literature.[3]

2.5 Synthesis of Mesylate 2



S4 **2**

The alcohol **S4** (100 mg, 471 µmol, 1 eq.) was dissolved in anhydrous THF (20 mL). Then, triethylamine (197 µL, 1.41 mmol, 3 eq.) was added to the yellow solution and the solution was cooled to 0 °C. After 10 min, mesyl chloride (54.7 µL, 707 µmol, 1.5 eq.) was added dropwise to the yellow solution at 0 °C. After full consumption of the starting material (monitored by TLC: EtOAc:hexanes: 1:1), the solvent was removed under reduced pressure to obtain a brown residue. The residue was redissolved in DCM (100 mL), and deionized water (50 mL) was added. The phases were separated, and the aqueous phase was extracted 3 times with DCM (3×100 mL). The combined organic phases were washed with brine and dried over sodium sulfate. After filtration, the organic phase was evaporated under reduced pressure. The brown residue was further purified with Biotage flash column chromatography (25 g SiO_2 cartridge, EtOAc:hexanes 10:90 to 100% EtOAc). The product **2** (120 mg, 413 µmol, 88%) was obtained as a beige solid. The product was stored in the fridge and was used within a few days due to its potential instability.

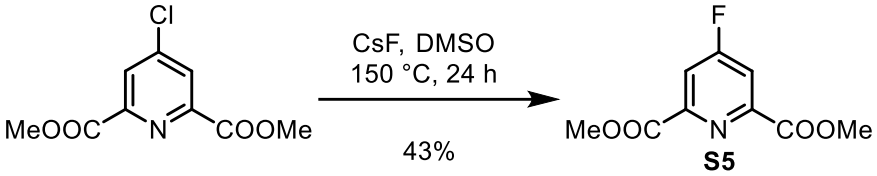
^{1}H NMR (500 MHz, $CDCl_3$, 298 K):
δ (ppm) = 8.77 (d, J = 2.0 Hz, 1 H, H_{arom}), 8.39 (d, J = 2.0 Hz, 1 H, H_{arom}), 5.55 (s, 2 H, CH_2), 4.08 (s, 3 H, CH_3), 3.22 (s, 3 H, CH_3).

$^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$, 298 K):
δ (ppm) = 163.5, 158.4, 155.8, 150.7, 117.8, 117.5, 69.4, 53.9, 38.3.

HR-DART-MS (positive, 200 °C):

$C_9H_{10}N_2O_7S$	calculated *m/z*	found *m/z*
$[M+H]^+$	291.0282	291.0286

2.6 Synthesis of Pyridine S5



Dimethyl 4-chloropyridine-2,6-dicarboxylate (1.00 g, 4.36 mmol, 1 eq.) was dissolved in anhydrous DMSO (5 mL). Then, cesium fluoride (860 mg, 5.66 mmol, 1.3 eq.), which was dried at 100 °C under high vacuum for 3 h, was added to the colorless solution. The colorless solution was degassed with nitrogen for 30 min and then heated at 150 °C with stirring for 24 h, resulting in a color change to dark brown. After allowing the reaction mixture to cool to room temperature, DCM (200 mL) and deionized water (100 mL) were added, and the aqueous phase was extracted 3 times with DCM (3×200 mL). The combined organic phases were washed with brine and dried over sodium sulfate. After filtration, the organic phase was evaporated under reduced pressure. The resulting brown oil was purified with Biotage flash column chromatography (50 g SiO_2 cartridge, EtOAc:hexanes 10:90 to 80:20). After evaporating the solvents, dimethyl 4-fluropyridine-2,6-dicarboxylate (**S5**) (402 mg, 1.89 mmol, 43%) was obtained as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$, 298 K):
δ (ppm) = 8.03 (d, J = 8.2 Hz, 2 H, H_{arom}), 4.04 (s, 4 H, CH_3).

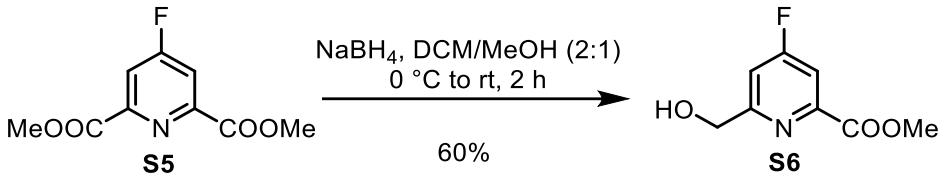
^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, 298 K):
δ (ppm) = 171.1, 169.0, 164.2, 164.2, 151.4, 151.3, 116.4, 116.2, 53.7.

^{19}F NMR (376 MHz, $CDCl_3$, 298 K):
δ (ppm) = –97.7 (t, J = 8.2 Hz).

HR-ESI-MS (MeCN):

$C_9H_8FNO_4$	calculated *m/z*	found *m/z*
$[M+H]^+$	214.0511	214.0511
$[M+Na]^+$	236.0330	236.0330
$[M+K]^+$	252.0069	252.0067

2.7 Synthesis of Alcohol S6



Dimethyl 4-fluoropyridine-2,6-dicarboxylate (**S5**) (400 mg, 1.88 mmol, 1 eq.) was dissolved in anhydrous DCM and methanol (2:1, 10 mL). The solution was cooled to 0 °C. Sodium borohydride (92.3 mL, 2.44 mmol, 1.3 eq.) was added portion wise over 60 min at 0 °C to the colorless solution, and the solution was then allowed to warm up to room temperature. After another hour at room temperature, full conversion of the starting material was achieved (monitored by TLC: EtOAc:hexanes: 1:1), and the solvents were removed under reduced pressure. DCM (200 mL) and deionized water (50 mL) were added to the resulting yellow oil, and the aqueous phase was extracted 3 times with DCM (3×150 mL). The combined organic phases were washed with brine and dried over sodium sulfate. After filtration, the organic phase was evaporated under reduced pressure, and the resulting brown oil was purified with Biotage flash column chromatography (25 g SiO_2 cartridge, EtOAc:hexanes 10:90 to 100%). The product **S6** (210 mg, 1.13 mmol, 60%) was obtained as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K):
δ (ppm) = 7.75 (dd, J = 8.7, 2.3 Hz, 1 H, H_{arom}), 7.32 (dd, J = 8.7, 2.4 Hz, 1 H, H_{arom}), 4.87 (s, 2 H, CH_2), 4.00 (s, 3 H, CH_3), 3.17 (s, 1 H, OH).

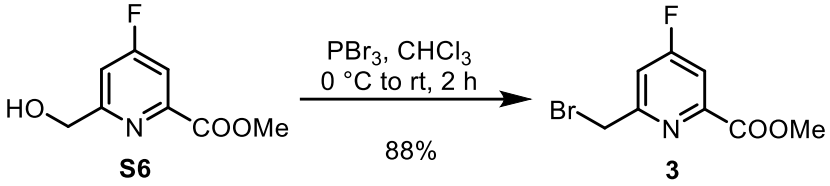
^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, 298 K):
δ (ppm) = 171.0, 168.9, 164.6, 164.1, 150.1, 112.3, 111.6, 64.7, 53.4.

^{19}F NMR (376 MHz, $CDCl_3$, 298 K):
δ (ppm) = –99.9 (t, J = 8.7 Hz).

HR-ESI-MS (MeCN):

$C_8H_8FNO_3$	calculated *m/z*	found *m/z*
$[M+H]^+$	186.0561	186.0563
$[M+Na]^+$	208.0381	208.0383
$[M+K]^+$	224.0120	224.0120

2.8 Synthesis of Pyridine 3



A solution of the alcohol **S6** (195 mg, 1.05 mmol, 1 eq.) in anhydrous chloroform (5 mL) was cooled to 0 °C. Phosphorus tribromide (110 µL, 1.16 mmol, 1.1 eq.) was added dropwise at 0 °C to the colorless solution. The reaction mixture was stirred for 1 h at 0 °C, then allowed to warm up to room temperature, and was continued stirring for another hour. After full conversion of the starting material (monitored by TLC: EtOAc:hexanes: 1:1), chloroform (200 mL) and deionized water (50 mL) were added to the yellow solution. The aqueous phase was separated and then extracted 3 times with chloroform (3×100 mL). The combined organic phases were washed with brine and dried over sodium sulfate. After filtration, the organic phase was evaporated under reduced pressure, and the resulting oil was further purified with Biotage flash column chromatography (25 g SiO_2 cartridge, EtOAc:hexanes 10:90 to 70:30). The product **3** (230 mg, 924 µmol, 88%) was obtained as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K):
δ (ppm) = 7.78 (d, J = 8.6 Hz, 1 H, H_{arom}), 7.43 (d, J = 8.6 Hz, 1 H, H_{arom}), 4.62 (s, 2 H, CH_2), 4.02 (s, 3 H, CH_3).

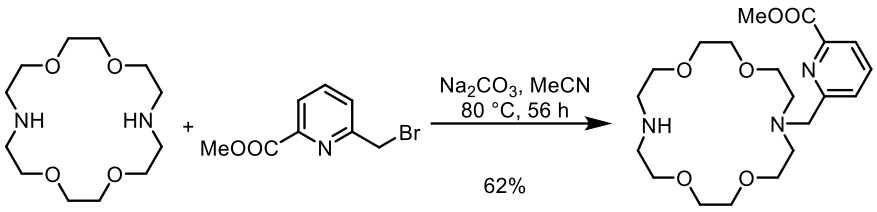
^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, 298 K):
δ (ppm) = 171.0, 168.8, 164.4, 160.7, 160.6, 150.6, 114.9, 114.8, 113.2, 113.0, 53.6, 32.5.

^{19}F NMR (376 MHz, $CDCl_3$, 298 K):
δ (ppm) = –99.2 (t, J = 8.9 Hz).

HR-DART-MS (positive, 200 °C):

$C_8H_7F^{79}Br^{81}BrNO_2$	calculated *m/z*	found *m/z*
$[M+H]^+$	247.9717	247.9710
	249.9697	249.9687

2.9 Synthesis of Crown Ether 1



Kryptofix® 22 **S7** **1**

Kryptofix® 22 (1.25 g, 4.77 mmol, 0.5 eq.) was dissolved in anhydrous acetonitrile (300 mL) and sodium carbonate (4.04 g, 38.1 mmol, 4 eq.) was added. The colorless suspension was heated to 80 °C. The functionalized pyridine **S7** (1.32 mg, 5.72 mmol, 0.6 eq.) was dissolved in anhydrous acetonitrile (200 mL) and added dropwise over 3 h to the crown ether suspension at 80 °C. After the addition of half of the pyridine solution into the crown ether solution, another portion of the crown ether (1.25 g, 4.77 mmol, 0.5 eq.) was added into the reaction mixture. After complete addition, the reaction mixture was stirred for 53 h at 80 °C. After full conversion of the pyridine starting material (monitored by TLC: EtOAc:hexanes: 1:1), the reaction mixture was filtered. The organic solvent was removed under reduced pressure, and the remaining yellow-brown oil was purified with Biotage flash column chromatography (25 g SiO_2 cartridge, DCM:MeOH 99:1 to 60:40). Fractions verified to be pure by TLC (DCM:MeOH: 9:1) were combined and concentrated to dryness. The resulting brown oil was redissolved in DCM and was filtered over a cotton wool. After removal of the solvent, the product **1** (1.46 g, 3.55 mmol, 60%) was obtained as a brown oil, which solidified over the course of 2 weeks.

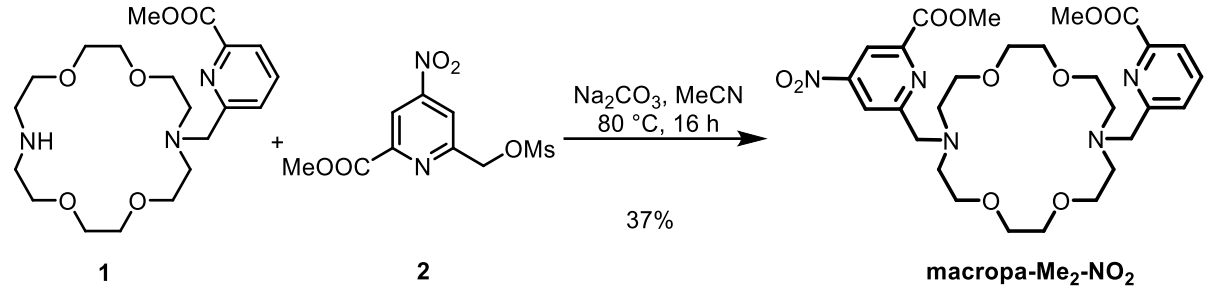
^{1}H NMR (400 MHz, CD_3OD, 298 K):
δ (ppm) = 8.06–7.97 (m, 2 H, H_{arom}), 7.82 (d, J = 7.7 Hz, 1 H, H_{arom}), 4.10 (s, 2 H, CH_2), 4.00 (s, 3 H, CH_3), 3.77 (t, J = 4.3 Hz, 4 H, CH_2), 3.67–3.58 (m, 8 H, CH_2), 3.55–3.48 (m, 4 H, CH_2), 3.36 (t, J = 5.2 Hz, 4 H, CH_2), 2.89 (t, J = 5.1 Hz, 4 H, CH_2).

^{13}C{^{1}H} NMR (126 MHz, CD_3OD, 298 K):
δ (ppm) = 167.1, 148.0, 139.7, 128.2, 124.8, 71.0, 70.6, 70.1, 66.7, 60.3, 55.7, 54.8, 53.5.

HR ESI-MS (MeOH):

$C_{20}H_{33}N_3O_6$	calculated *m/z*	found *m/z*
$[M+H]^+$	412.2443	412.2403
$[M+Na]^+$	434.2262	434.2220
$[M+K]^+$	450.2001	450.1958
$[M+2H]^{2+}$	206.6258	206.6239

2.10 Synthesis of Crown Ether Macropa-Me_2-NO_2



1 **2** **macropa-Me_2-NO_2**

The crown ether **1** (89.3 mg, 217 µmol, 1 eq.) was dissolved in anhydrous acetonitrile (8 mL), and sodium carbonate (69.0 mg, 651 µmol, 3 eq.) was added. The colorless suspension was heated to 80 °C for 30 min, and then a solution of mesylate **2** (63.0 mg, 217 mmol, 1 eq.) in anhydrous acetonitrile (2 mL) was added. The reaction mixture was stirred for 16 h at 80 °C, after which full conversion was achieved (monitored by TLC: DCM:MeOH 9:1). The reaction mixture was filtered, and the organic solvent was concentrated to dryness under vacuum. The remaining yellow-brown oil was purified with Biotage flash column chromatography (25 g SiO_2 cartridge, DCM:MeOH 99:1 to 85:15). Fractions verified to be pure by TLC (DCM:MeOH: 9:1) were combined, and the solvent was removed under reduced pressure. The resulting brown oil was dissolved in DCM and was filtered over a cotton wool. After removal of the solvent, the product **macropa-Me_2-NO_2** (99.9 mg, 165 µmol, 76%) was obtained as a yellow oil. This yellow oil was not sufficiently pure, as determined by NMR spectroscopy. It was subsequently subject to further purification via preparative HPLC (*system 1*) to yield **macropa-Me_2-NO_2** as a brown oil and a TFA salt (88 mg, 80.2 µmol, 37%).

^{1}H NMR (400 MHz, CD_3OD, 298 K):
δ (ppm) = 8.77 (s, 1 H, H_{arom}), 8.53 (s, 1 H, H_{arom}), 8.20 (d, J = 7.8 Hz, 1 H, H_{arom}), 8.13 (t, J = 7.6 Hz, 1 H, H_{arom}), 7.76 (d, J = 7.7 Hz, 1 H, H_{arom}), 4.97 (s, 2 H, CH_2), 4.80 (s, 2 H, CH_2), 4.08 (s, 3 H, CH_3), 4.03 (s, 3 H, CH_3), 4.03–3.93 (m, 8 H, CH_2), 3.86–3.55 (m, 16 H, CH_2).

^{13}C{^{1}H} NMR (126 MHz, CD_3OD, 298 K):
δ (ppm) = 166.4, 164.8, 157.4, 155.6, 152.2, 151.3, 148.6, 140.8, 128.7, 126.5, 121.8, 119.2, 71.5, 71.4, 65.9 (2 C), 58.6, 58.3, 55.5 (2 C), 54.1, 53.6.

^{19}F NMR (376 MHz, CD_3OD, 298 K):
δ (ppm) = –77.4 (CF_3COOH).

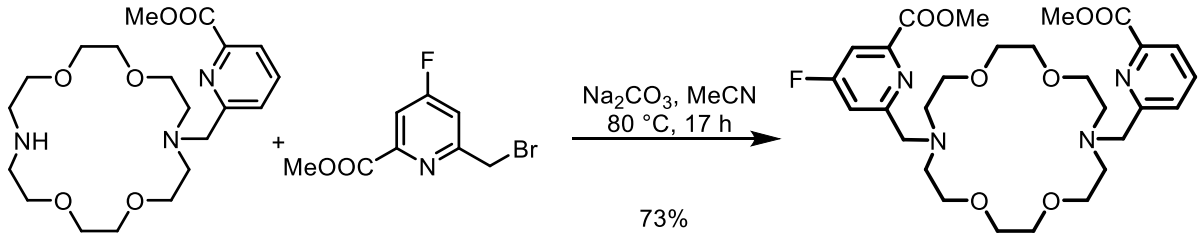
HR-ESI-MS (MeOH):

$C_{28}H_{39}N_5O_{10}$	calculated *m/z*	found *m/z*
$[M+H]^+$	606.2770	606.2734
$[M+Na]^+$	628.2590	628.2540
$[M+K]^+$	644.2329	644.2287
$[M+2H]^{2+}$	303.6421	303.6404

Elemental Analysis: macropa-NO_2·4TFA·$2H_2O$

	C	**H**	**N**
Calculated [%]	39.39	4.32	6.38
Found [%]	39.29	4.24	6.32

2.11 Synthesis of Crown Ether 4



1 **3** **4**

The crown ether **1** (170 mg, 413 µmol, 1 eq.) was dissolved in anhydrous acetonitrile (10 mL), and sodium carbonate (131 mg, 1.24 mmol, 3 eq.) was added. The colorless suspension was heated at 80 °C for 30 min, after which a solution of the brominated pyridine **3** (113 mg, 454 mmol, 1 eq.) in anhydrous acetonitrile (10 mL) was added over the course of 5 min. After complete addition, the reaction mixture was stirred for 17 h at 80 °C. After full conversion (monitored by TLC: DCM:MeOH 9:1), the reaction mixture was filtered, and the filtrate was concentrated to dryness under reduced pressure. The remaining yellow-brown oil was purified with Biotage flash column chromatography (25 g SiO_2 cartridge, DCM:MeOH 99:1 to 85:15). Fractions verified to be pure by TLC (DCM:MeOH: 9:1) were combined and concentrated to dryness. The brown oil was dissolved in DCM and filtered through cotton wool. After removal of the solvent, the product **4** was obtained as a brown oil. To achieve higher chemical purity, the oil was further purified with Biotage column chromatography equipped with a C_{18}-functionalized reverse-phase cartridge (10 g SiO_2 cartridge, H_2O+0.1% TFA:MeOH 95:5 to 0:65). The collected fractions were analyzed via analytical HPLC (*system 2*), and fractions with the same retention time and high chemical purity

were combined. The fractions were concentrated and lyophilized. The crown ether **4** was obtained as a colorless oil after lyophilization (174 mg, 301 mmol, 73%).

^{1}H NMR (400 MHz, CD_3OD, 298 K):
δ (ppm) = 8.19 (d, J = 7.7 Hz, 1 H, H_{arom}), 8.12 (t, J = 6.7 Hz, 1 H, H_{arom}), 7.98 (d, J = 8.7 Hz, 1 H, H_{arom}), 7.75 (d, J = 7.5 Hz, 1 H, H_{arom}), 7.63 (dd, J = 8.3 Hz, 1 H, H_{arom}), 4.79 (s, 4 H, CH_2), 4.03 (s, 6 H, CH_3), 3.97 (s, 8 H, CH_2), 3.70 (s, 16 H, CH_2).

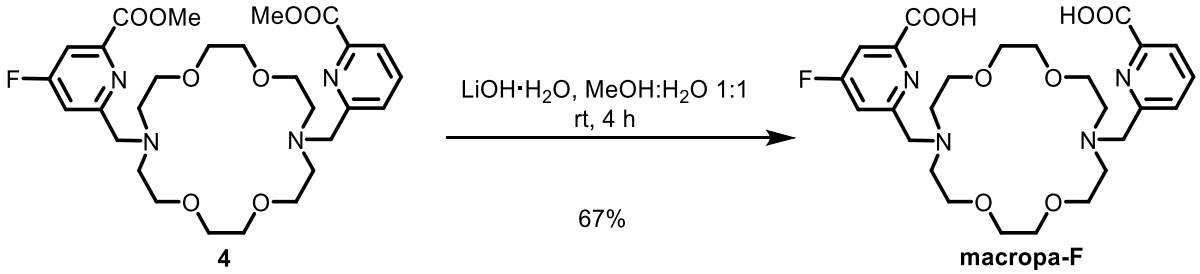
^{13}C{^{1}H} NMR (126 MHz, CD_3OD, 298 K):
δ (ppm) = 172.7, 170.6, 166.4, 165.4, 155.5, 152.2, 151.7, 148.6, 140.8, 128.7, 126.5, 116.5, 114.8, 71.5 (2 C), 65.9, 58.7, 58.4 (2 C), 55.5 (2 C), 53.9, 53.6.

^{19}F NMR (376 MHz, CD_3OD, 298 K):
δ (ppm) = –77.1 (CF_3COOH), –99.8 (t, J = 7.8 Hz).

HR-ESI-MS (MeOH):

$C_{28}H_{39}N_4O_8$	calculated *m/z*	found *m/z*
$[M+H]^+$	579.2825	579.2808
$[M+Na]^+$	601.2645	601.2625
$[M+K]^+$	617.2384	617.2367

2.12 Synthesis of the Chelator Macropa-F



4 **macropa-F**

The crown ether **4** (52 mg, 89.9 µmol, 1 eq.) was dissolved in methanol (5 mL), and a solution o lithium hydroxide monohydrate (7.73 mg, 189 µmol, 2.1 eq.) dissolved in deionized water (5 mL) was added. The yellow solution was stirred for 4 h at room temperature, and the progress of the reaction was monitored via analytical HPLC (*system 2*). After complete deprotection, the volume of the reaction mixture was reduced by half, and an aqueous solution of hydrochloric acid (0.5 M) was added to adjust the pH to 5. The solution was then lyophilized, and the remaining residue was purified with Biotage column chromatography equipped with a C_{18}-functionalized reverse-phase cartridge (10 g SiO_2 cartridge, H_2O+0.1% TFA:MeOH 95:5 to 0:100). The fractions were analyzed via analytical HPLC (*system 2*), and fractions with the same retention time and high

purity were combined. The fractions were concentrated and lyophilized. After lyophilization, the chelator **macropa-F** was obtained as a colorless and hygroscopic TFA salt (51.0 mg, 60.2 µmol, 67%). Attempts to convert the TFA salt to the HCl salt by using an aqueous solution of hydrochloric acid (0.1 N) led to the substitution of the aromatic fluorine with chlorine. For this reason, the chelator was kept as a TFA salt.

^{1}H NMR (400 MHz, CD_3OD, 298 K):
δ (ppm) = 8.21 (d, J = 6.7 Hz, 1 H, H_{arom}), 8.12 (t, J = 7.8 Hz, 1 H, H_{arom}), 7.97 (dd, J = 8.8, 2.3 Hz, 1 H, H_{arom}), 7.73 (d, J = 7.8 Hz, 1 H, H_{arom}), 7.59 (dd, J = 8.4, 2.3 Hz, 1 H, H_{arom}), 4.79 (s, 4 H, CH_2), 3.95 (s, 8 H, CH_2), 3.68 (s, 16 H, CH_2).

^{13}C{^{1}H} NMR (126 MHz, D_2O, 298 K):
δ (ppm) = 173.8, 172.3, 171.6, 169.5, 163.8, 163.5, 162.1, 158.0, 157.1, 153.8, 139.0, 126.4, 122.9, 118.1, 115.8, 113.6, 110.9, 70.0, 68.9, 60.6, 60.3, 54.2, 53.9, 1.5.

^{19}F NMR (376 MHz, CD_3OD, 298 K):
δ (ppm) = –77.1 (CF_3COOH), –99.8 (t, J = 7.8 Hz).

HR-ESI-MS (MeOH):

$C_{26}H_{35}FN_4O_8$	calculated *m/z*	found *m/z*
$[M+H]^+$	551.2512	551.2534
$[M+Na]^+$	573.2332	573.2353
$[M+K]^+$	589.2071	589.2017
$[M+2H]^+$	276.1292	276.1304

Elemental Analysis: macropa-F·2TFA·2H_2O·MeOH

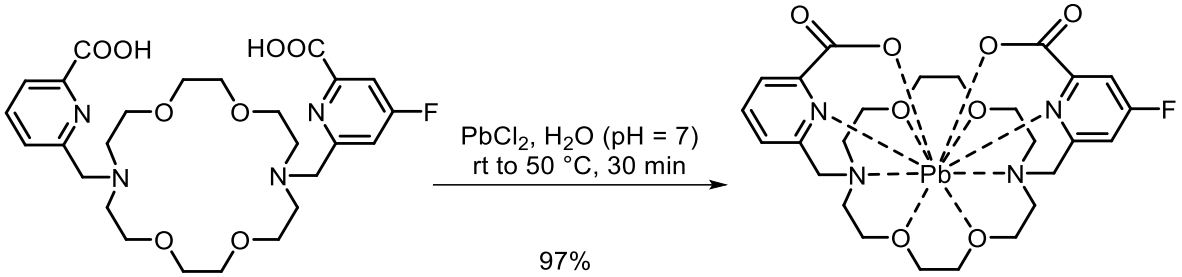
	C	**H**	**N**	**F**
Calculated [%]	43.98	5.36	6.62	15.71
Found [%]	44.25	4.99	7.00	15.31

3. Metal Complex Formation Experiments

The chelators **macropa-F** or **macropa-NO_2** (1 eq.) were dissolved in deuterated water. The pD of the chelator solution was adjusted to pD = 7 by using a solution of sodium deuteroxide (0.1 N) for lanthanum, lead and lutetium and to pD = 5 for bismuth, respectively. In a separate vial, the metal salt ($LaCl_3·7H_2O$, $Pb(ClO_4)_2$, $Bi(NO_3)_3·5H_2O$, and $LuCl_3$) (1.1 eq.) was dissolved in deuterated water, and the pD was adjusted to pD = 7 for the lanthanum and the lead salt and to pD = 5 for the bismuth salt. Then, the pD-adjusted chelator and metal solutions were combined,

and were filtered over a PTFE-syringe filter (0.45 µm), and the solutions were heated to 50 °C for 10 min. After this time, acetonitrile (300 µL) as chemical shift reference standard was added to each metal complexation reaction, and the solutions were analyzed via ^{1}H NMR spectroscopy.

3.1 Synthesis of [Pb(macropa-F)]



macropa-F **[Pb(macropa-F)]**

Following the procedure described above, the lead complex [Pb(macropa-F)] was synthesized using macropa-F (5 mg, 5.91 µmol, 1 eq.) and $PbCl_2$ (1.81 mg, 6.50 µmol, 1.1 eq.) in deuterated water (pD = 7). The reaction solution was stirred for 30 min at 50 °C. Then, the solution was filtered over a PTFE-syringe filter (0.45 µm), and the remaining colorless solution was lyophilized. The colorless solid (4.33 mg, 5.73 µmol, 97%) was characterized via NMR spectroscopy and HR-ESI-MS.

^{1}H NMR (500 MHz, D_2O, 298 K):
δ (ppm) = 7.94 (t, J = 7.8 Hz, 1 H, H_{arom}), 7.64 (d, J = 7.5 Hz, 1 H, H_{arom}), 7.53 (d, J = 7.8 Hz, 1 H, H_{arom}), 7.38 (d, J = 6.1 Hz, 1 H, H_{arom}), 7.20 (d, J = 7.9 Hz, 1 H, H_{arom}), 5.11 (d, J = 16.3 Hz, 2 H, CH_2), 4.41 (d, J = 11.0 Hz, 2 H, CH_2), 3.92 (d, J = 9.2 Hz, 2 H, CH_2), 3.86–3.63 (m, 10 H, CH_2), 3.57 (d, J = 8.5 Hz, 2 H, CH_2), 3.46 (d, J = 10.1 Hz, 2 H, CH_2), 3.29 (s, 2 H, CH_2), 2.59 (d, J = 10.1 Hz, 4 H, CH_2), 2.45 (d, J = 11.6 Hz, 2 H, CH_2).

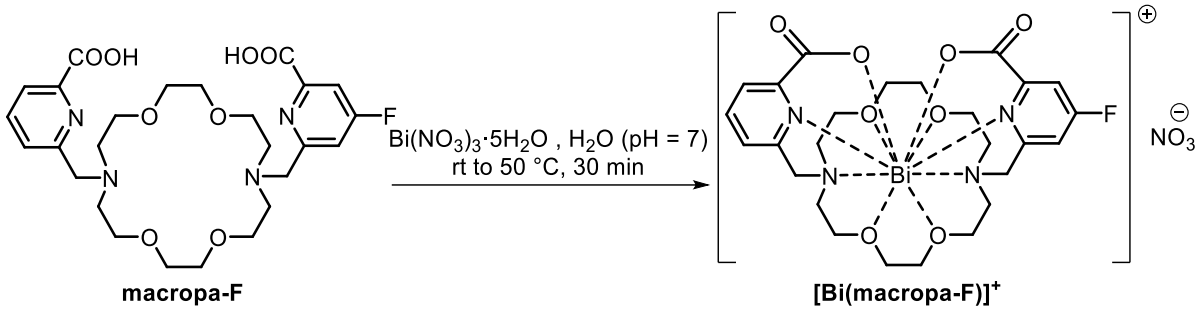
^{13}C{^{1}H} NMR (126 MHz, D_2O, 298 K):
δ (ppm) = 172.8, 171.9, 171.1, 169.8, 164.5, 164.1, 163.8, 163.5, 163.3, 159.6, 154.7, 150.5, 140.6, 127.6, 123.8, 120.5, 118.2, 115.9, 115.0, 114.9, 113.5, 112.2, 112.1, 70.7, 70.1, 69.0, 67.8, 60.0, 59.9, 55.3, 54.1, 54.0.

^{19}F NMR (376 MHz, D_2O, 298 K):
δ (ppm) = –75.6 ($C\underline{F}_3COOH$), –97.1 (t, J = 8.5 Hz).

HR-ESI-MS (MeOH):

$C_{26}H_{33}FN_4O_8Pb$	calculated *m/z*	found *m/z*
$[M+H]^+$	757.2122	757.2119

3.2 Synthesis of [Bi(macropa-F)]$^+$



macropa-F **[Bi(macropa-F)]$^+$**

Following the procedure described above, the bismuth complex [Bi(macropa-F)]$^+$ was synthesized using macropa-F (5 mg, 5.91 µmol, 1 eq.) and $Bi(NO_3)_3 \cdot 5H_2O$ (3.15 mg, 6.50 µmol, 1.1 eq.) in deuterated water (pD = 5). The resulting solution was stirred for 30 min at 50 °C. A colorless solid precipitated, and this suspension was filtered over a PTFE-syringe filter (0.45 µm). The remaining colorless solution was lyophilized. The colorless solid (3.88 mg, 4.73 µmol, 80%) was characterized via NMR spectroscopy and HR-ESI-MS.

^{1}H NMR (500 MHz, D_2O, 298 K):
δ (ppm) = 8.31 (t, J = 7.8 Hz, 1 H, H_{arom}), 8.05 (d, J = 7.6 Hz, 1 H, H_{arom}), 7.99 (d, J = 7.8 Hz, 1 H, H_{arom}), 7.77 (d, J = 5.6 Hz, 1 H, H_{arom}), 7.70 (d, J = 8.1 Hz, 1 H, H_{arom}), 4.46 (dd, J = 16.5, 8.9 Hz, 2 H, CH_2), 4.39 (d, J = 16.8 Hz, 1 H, CH_2), 4.28 (d, J = 16.6 Hz, 1 H, CH_2), 3.90–3.80 (m, 1 H, CH_2), 3.79–3.67 (m, 3 H, CH_2), 3.67–3.54 (m, 3 H, CH_2), 3.54–3.44 (m, 1 H, CH_2), 3.44–3.31 (m, 4 H, CH_2), 3.33–3.23 (m, 1 H, CH_2), 3.22–3.12 (m, 1 H, CH_2), 3.10–2.91 (m, 4 H, CH_2), 2.80–2.58 (m, 4 H, CH_2), 2.58–2.46 (m, 1 H, CH_2), 2.38–2.27 (m, 1 H, CH_2).

^{13}C{^{1}H} NMR (126 MHz, D_2O, 298 K):
δ (ppm) = 173.6, 171.5, 171.1, 169.5, 164.1, 163.8, 163.6, 159.2, 153.3, 153.2, 148.8, 144.1, 128.7, 126.8, 120.7, 120.0, 118.4, 116.7, 116.5, 116.1, 115.7, 115.6, 69.7, 69.5, 69.3, 69.0, 68.4, 68.3, 65.8, 65.5, 62.3, 61.8, 56.8, 56.2, 56.0, 55.7.

^{19}F NMR (376 MHz, D_2O, 298 K):
δ (ppm) = –75.6 ($C\underline{F}_3COOH$), –89.8 (t, J = 7.8 Hz).

HR-ESI-MS (MeOH):

$C_{26}H_{33}FN_4O_8Bi^+$	calculated *m/z*	found *m/z*
$[M]^+$	757.2081	757.2059

3.3 NMR Spectra of Macropa-NO_2 with Different Metal Ions

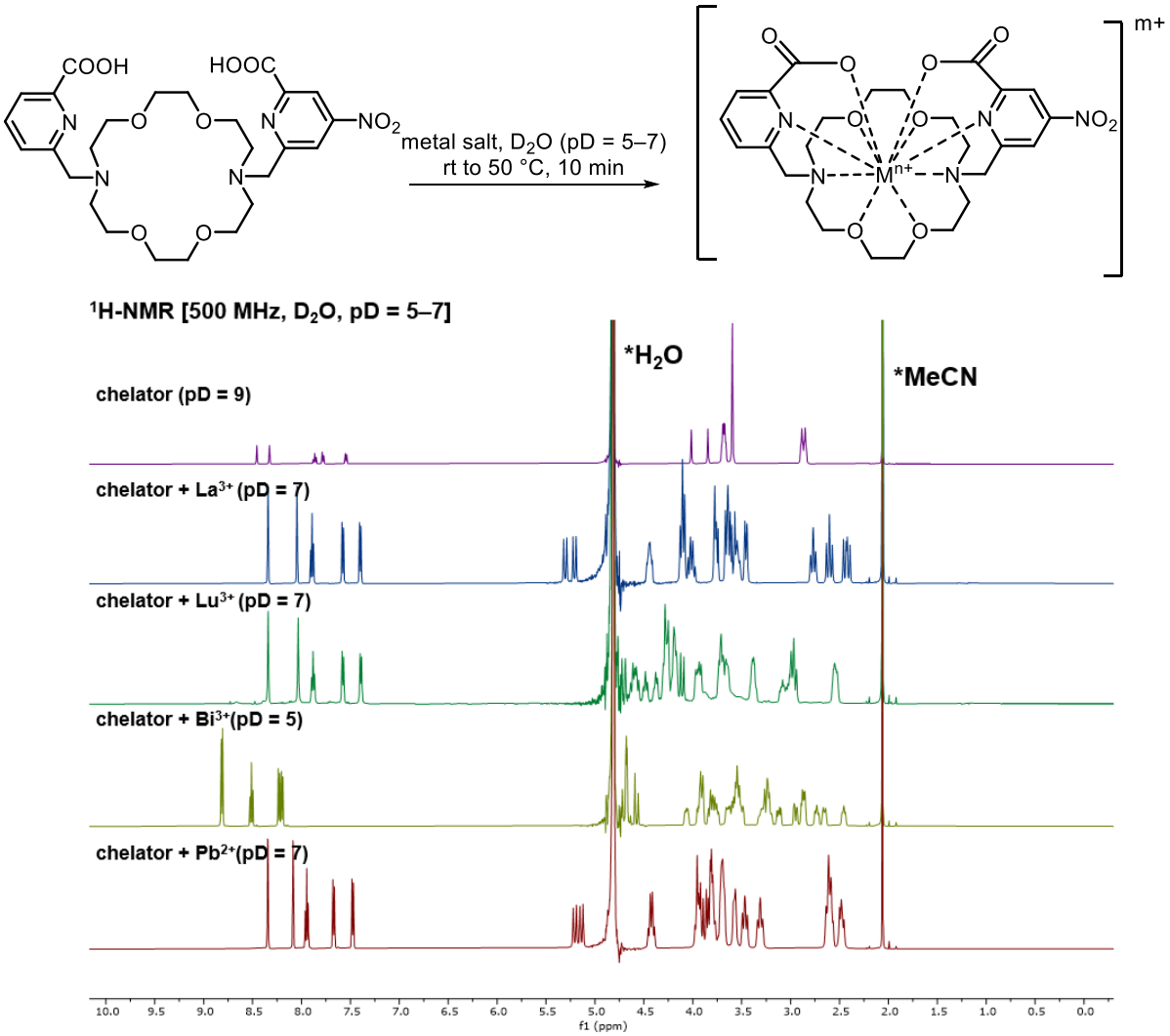


Figure S1: ^{1}H NMR spectra of **macropa-NO_2** in pD-adjusted deuterated solution in the presence of La^{3+}, Lu^{3+}, Bi^{3+}, and Pb^{2+}. Acetonitrile was used as reference standard solution.

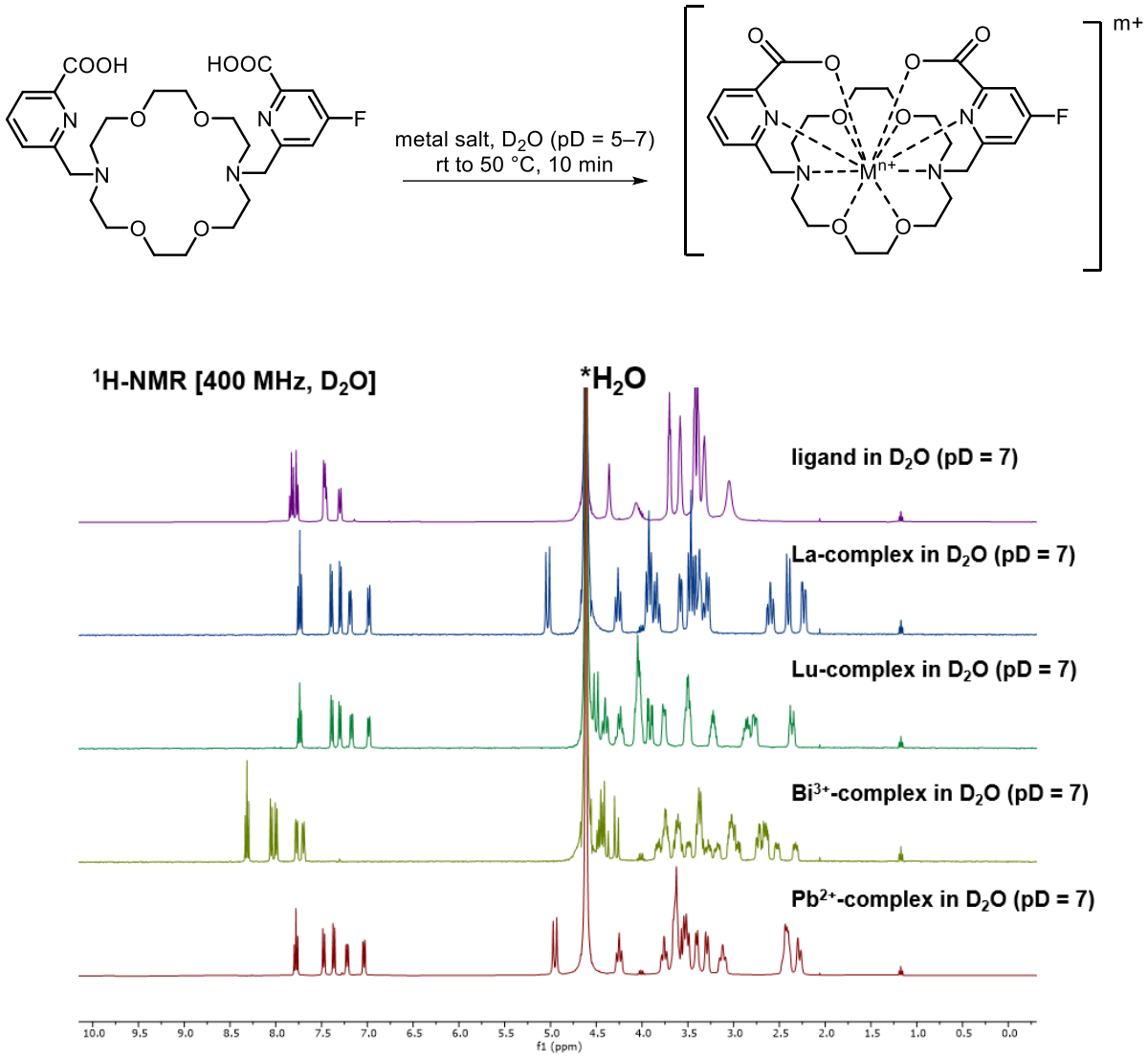


Figure S2: ^{1}H NMR spectra of **macropa-F** in pD-adjusted deuterated solution in the presence of La^{3+}, Lu^{3+}, Bi^{3+}, and Pb^{2+}. Acetonitrile was used as reference standard solution.

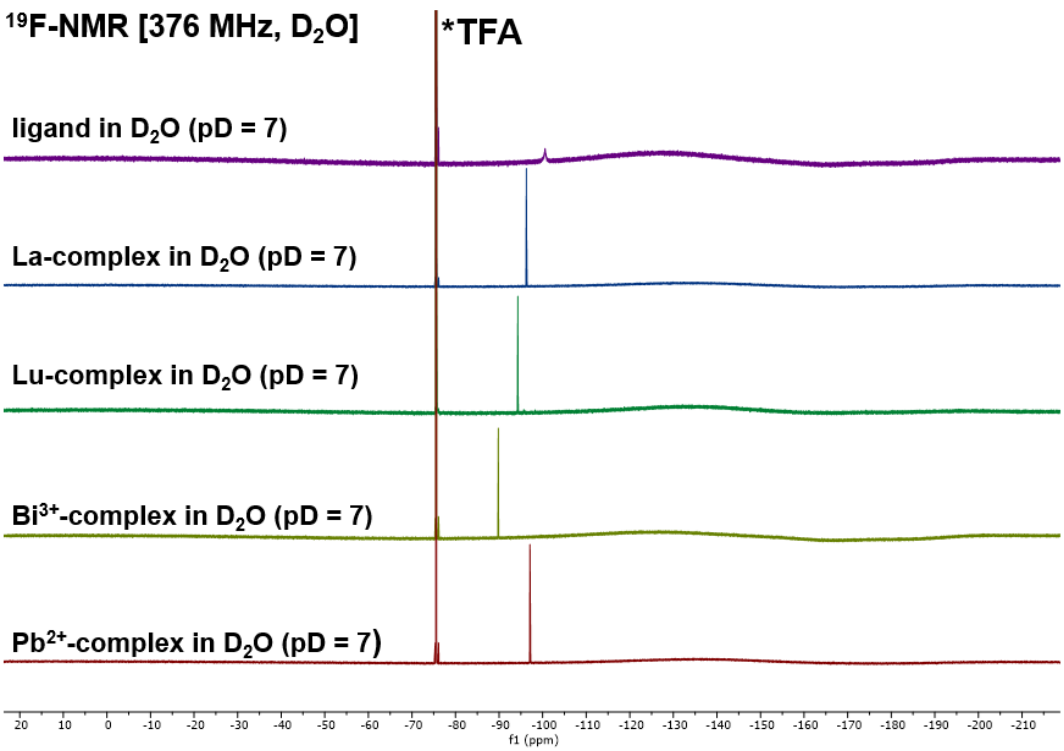


Figure S3: ^{19}F NMR spectra of **macropa-F** in pD-adjusted deuterated solution in the presence of La^{3+}, Lu^{3+}, Bi^{3+}, and Pb^{2+}. Acetonitrile was used as reference standard solution.

4. Crystal Structure

Suitable X-ray crystals for X-ray diffraction studies of $[Bi(macropa\text{-}F)]^+$ were obtained via the following procedure. To obtain the $[Bi(macropa\text{-}F)]^+$ complex, macropa-F (5 mg, 5.91 µmol, 1 eq.) and $Bi(NO_3)_3 \cdot 5H_2O$ (3.15 mg, 6.50 µmol, 1.1 eq.) were dissolved in deionized water in separate vials (100 µL). Then, the pH of both solutions was adjusted to ca. 5 by using an aqueous solution of sodium hydroxide (0.1 N). The metal- and ligand-containing solutions were combined and a precipitation occurred. The resulting metal complex was heated to 50 °C for 10 min. Afterwards, the solvent was removed by using a gentle air flush. Then, the colorless residue was redissolved in methanol and was filtered over a PTFE-syringe filter (0.45 µm). Yellow crystals were obtained within one week by slow vapor diffusion of diethyl ether into a methanolic solution containing the metal complex at –20 °C.

Low-temperature X-ray diffraction data for the $[Bi(macropa\text{-}F)]^+$ were collected on a Rigaku XtaLAB Synergy diffractometer coupled to a Rigaku Hypix detector with Mo K_α radiation source (λ = 0.71073 Å), from a PhotonJet micro-focus X-ray source at 100 K. The diffraction images were processed and scaled using the CrysAlisPro software.[4] The structures were solved through intrinsic phasing using SHELXT and refined against F2 on all data by full-matrix least squares with SHELXL following established refinement strategies.[5-7] All non-hydrogen atoms were refined

anisotropically. All hydrogen atoms bound to carbon were included in the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U_{eq} value of the atoms they are linked to (1.5 times for methyl groups). Details of the data quality and a summary of the residual values of the refinements are listed in Table S1. In addition, selected interatomic distances between the donor atoms and the metal center are shown in Table S2. Crystallographic data for $[Bi(macropa\text{-}F)]^+$ in the form of a .cif file have been deposited in the Cambridge Crystallographic Structural Database (CCDC) under accession number CCDC 2377788.

Table S1: Crystal Data and Structure Refinement for $[Bi(macropa\text{-}F)]^+$.

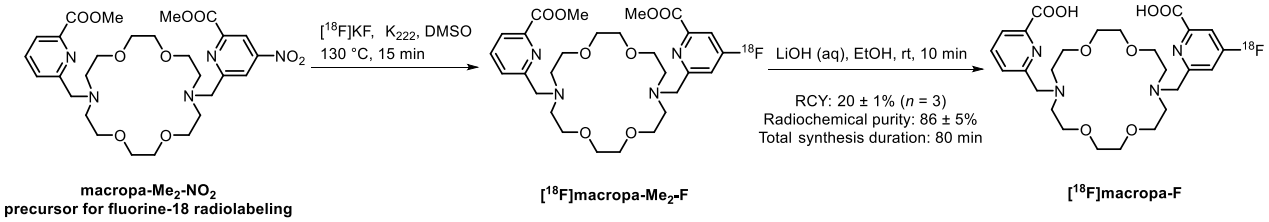
Empirical formula	$C_{27}H_{36}BiFN_6NaO_{15}$
Formula weight	935.59
Temperature	108.29(10) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions	a = 10.17370(10) Å b = 12.42250(10) Å c = 15.0689(2) Å α = 68.9380(10)° β = 85.2210(10)° γ = 79.1070(10)°
Volume	1744.95(3) $Å^3$
Z	2
Density (calculated)	1.781 Mg/m^3
Absorption coefficient	5.147 mm^{-1}
F(000)	926
Crystal size	0.277 × 0.084 × 0.066 mm^3
Theta range for data collection	2.469 to 27.103°
Index ranges	-13<=h<=12, -15<=k<=15, -19<=l<=19
Reflections collected	74691
Independent reflections	7683 [R(int) = 0.0488]
Completeness to theta = 25.242°	99.9%
Absorption correction	Gaussian
Max. and min. transmission	1.000 and 0.402
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	7683 / 47 / 516
Goodness-of-fit on F^2	1.048
Final R indices [I>2sigma(I)]	R1 = 0.0192, wR2 = 0.0442
R indices (all data)	R1 = 0.0218, wR2 = 0.0450
Extinction coefficient	n/a
Largest diff. peak and hole	0.982 and -0.663 $e.Å^{-3}$

Table S2: Interatomic Distances (Å) of Trivalent Bismuth with Macropa and Macropa-F.

	[Bi(macropa)]$^{+}$, [8]	**[Bi(macropa-F)]$^{+}$**
$Bi–O_{carboxy}$	2.2577(16)	2.251(2)
$Bi–O_{carboxy}$	2.2316(17)	2.266(2)
$Bi–O_{crown}$	2.7541(17)	2.749(2)
$Bi–O_{crown}$	3.048(2)	3.090(2)
$Bi–O_{crown}$	2.987(2)	2.925(2)
$Bi–O_{crown}$	2.895(2)	2.899(2)
$Bi–N_{py}$	2.4419(19)	2.453(2)
$Bi–N_{crown}$	2.930(1)	2.925(2)
$Bi–N_{crown}$	2.688(2)	2.684(2)
$Bi–N_{py}$	2.413(2)	2.403(2)

5. Radiolabelling of Macropa Precursor Macropa-Me_2-NO_2 with Fluorine-18

5.1 Synthesis of [^{18}F]macropa-F



[^{18}F]macropa-F was synthesized using a TRACERlab Fx2N synthesizer from GE Healthcare Technologies Inc. In particular, the mononitro functionalized precursor (**macropa-Me_2-NO_2**, 8 mg, 13 µmol) in DMSO (0.3 mL) was added to an azeotropically dried mixture of [^{18}F]KF/K_{222} (K_2CO_3: 12 mg, K_{222}: 15 mg, ~15 GBq) and was heated at 130 °C for 15 min. After cooling to room temperature, the mixture was treated with lithium hydroxide (0.2 M, 250 µL) and ethanol (250 µL), stirred for 10 min, and then neutralized with an aqueous solution of hydrochloric acid (0.1 M, 1 mL). The reaction mixture was diluted with deionized water with 0.1% v/v acetic acid. The crude solution was purified by semi-preparative HPLC (*system 4*). The RCC from the first radiolabelling step was 69 ± 12% (n = 4), determined by analytical HPLC. The isolated RCY was calculated to be 20 ± 1% (n = 3). The radiochemical purity of [^{18}F]macropa-F was 86 ± 5%.

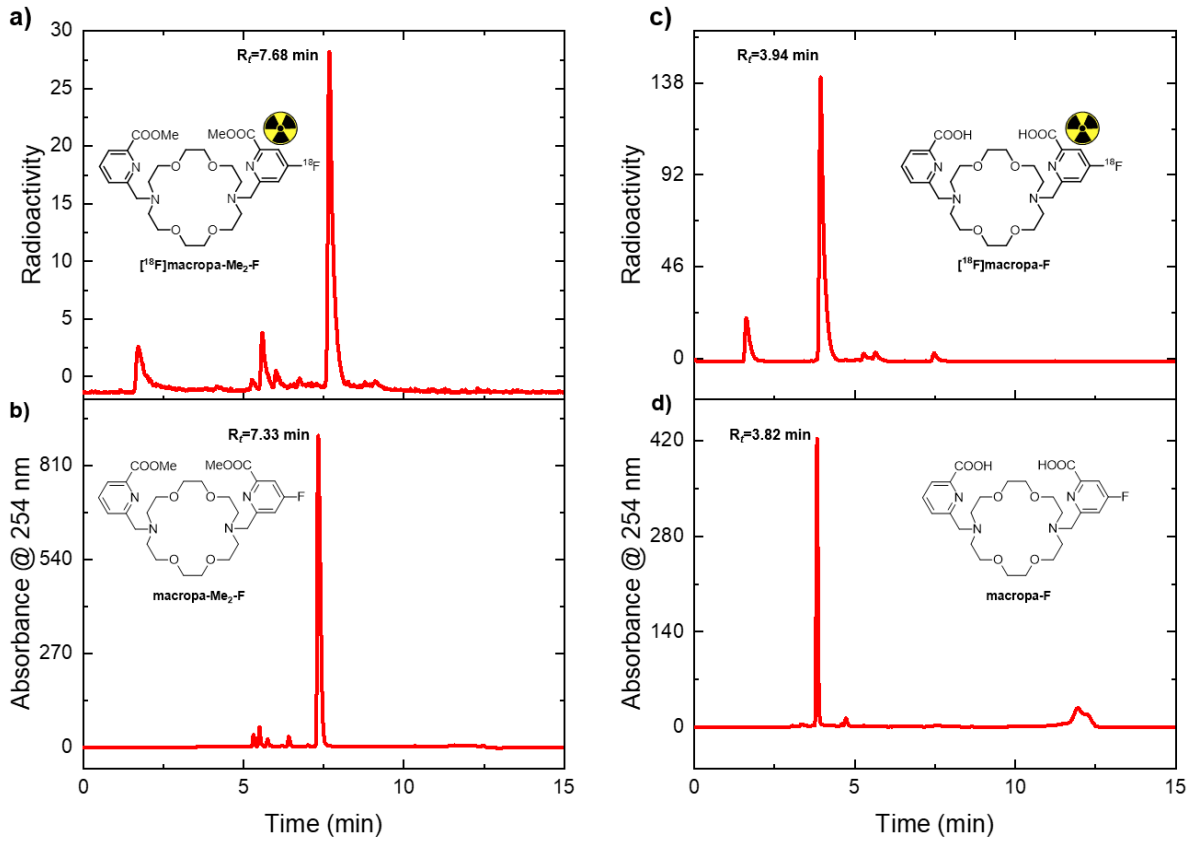
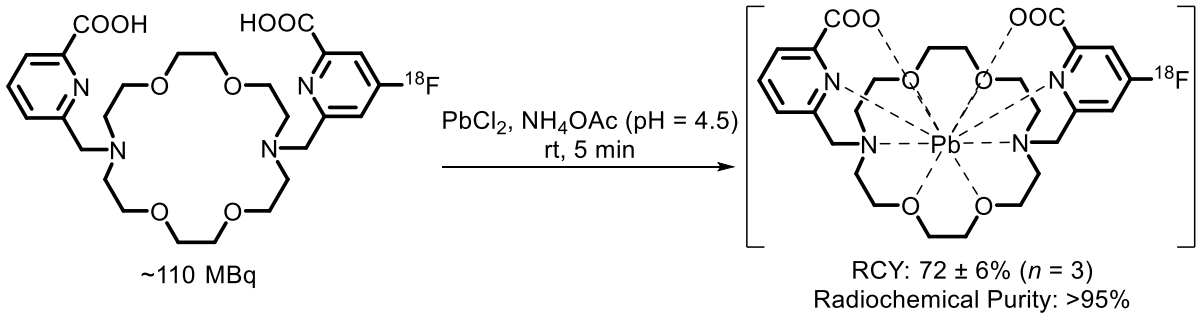


Figure S4: a) Radio-HPLC chromatograms of radiofluorinated **[^{18}F]macropa-Me$_2$-F** and b) the UV-vis HPLC chromatogram of the non-radioactive reference standard **macropa-Me$_2$-F**. c) Radio-HPLC trace of the radiofluorinated **[^{18}F]macropa-F** and d) the UV-vis HPLC chromatogram of the non-radioactive reference standard **macropa-F** (HPLC method: *system 5*).

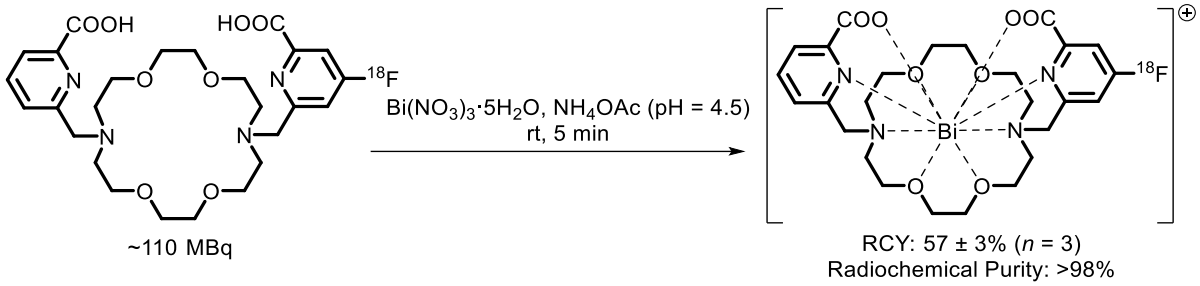
5.2 Metal Complexation of [^{18}F]macropa-F with PbCl$_2$



After HPLC purification, the fraction containing [^{18}F]macropa-F (~110 MBq) solution was combined with solid PbCl$_2$ (2 mg, 7.19 µmol) in ammonium acetate buffer (pH 4.5, 10 mM, 10 mL). The mixture was stirred for 5 min at room temperature. Subsequently, the solution was loaded onto a C18 cartridge. Then, the product on the cartridge was washed with a solution of

NH_4OAc buffer (pH = 4.5, 10 mM, 10 mL). Afterwards, the product was eluted with a solution of DMSO (0.6 mL) and NH_4OAc buffer (pH 4.5, 10 mM, 2.4 mL). [^{18}F][Pb(macropa-F)] was obtained with a RCY of 73 ± 6% (*n* = 3) and a radiochemical purity exceeding 95%. The proof-of-identity and the purity were evaluated by comparison of retention times with the non-radioactive reference [Pb(macropa-F)] (HPLC method: *system 5*).

5.3 Metal Complexation of [^{18}F]macropa-F with $Bi(NO_3)_3 \cdot 5H_2O$



After HPLC purification, the fraction containing the [^{18}F]macropa-F (~110 MBq) solution was combined with solid $Bi(NO_3)_3 \cdot 5H_2O$ (2 mg, 4.12 µmol) in ammonium acetate buffer (pH 4.5, 10 mM, 10 mL). The mixture was stirred for 5 min at room temperature. Subsequently, the solution was loaded onto a C18 cartridge. Then, the product on the cartridge was washed with a solution of NH_4OAc buffer (pH 4.5, 10 mM, 10 mL). Afterwards, the product was eluted with a solution of DMSO (0.6 mL) and NH_4OAc buffer (pH = 4.5, 10 mM, 2.4 mL). [^{18}F][Bi(macropa-F)]$^+$ was obtained with a RCY of 57 ± 3% (*n* = 3) and a radiochemical purity exceeding 98%. The proof-of-identity and the purity were evaluated by comparison of retention times with the non-radioactive reference [Bi(macropa-F)]$^+$ (HPLC method: *system 5*).

5.4 Human Serum Stability Studies of [^{18}F][Pb(macropa-F)] and [^{18}F][Bi(macropa-F)]$^+$

Human serum stability studies were performed as previously reported with minor modifications.[9] The radioactive and reformulated complexes of [^{18}F][Pb(macropa-F)] or [^{18}F][Bi(macropa-F)]$^+$ (~3.7 MBq, 50 µL) were incubated as solutions in human serum (250 µL, Sigma-Aldrich) at 37 °C. After several timepoints (0, 0.5, 1, 2, and 4 h), aliquots (50 µL) of the radioactive solution were taken at post-incubation. Then, the plasma proteins were precipitated by the addition of methanol (100 µL) to each aliquot. After centrifugation, the supernatants were analyzed by HPLC. The process was repeated in triplicate.

Table S3: Human Serum Stability Studies of [^{18}F][Pb(macropa-F)] or [^{18}F][Bi(macropa-F)]$^{+}$ as Analyzed by Radio-HPLC (Experiments were Performed in Triplicate).

	[^{18}F][Pb(macropa-F)]	**[^{18}F][Bi(macropa-F)]$^{+}$**
Time	Intact Complex [%]	Intact Complex [%]
0 min	>95%	>98%
30 min	>95%	>98%
1 h	>95%	>98%
2 h	>95%	>98%
4 h	>95%	>98%

6. Radiolabelling of Macropa and Macropa-F with [^{203}Pb]Pb^{2+}

Radioactive lead-203 was purchased from the U.S. Department of Energy Isotope Program, which was produced at the University of Alabama at Birmingham (UAB) Cyclotron Facility in a Tl-205(p,3n)Pb-203 reaction with a radiochemical purity of >98%. The initial activity of the lead-203 was 5.20 mCi (192.4 MBq) which was dissolved in aqueous hydrochloric acid (1 M, 11 µL). The radioactivity was diluted with aqueous hydrochloric acid (1 M, 189 µL) (prepared with ultra pure water and trace metal grade hydrochloric acid, total radioactivity volume: 200 µL) for the concentration-dependent radiolabelling studies. For the human serum stability studies, the radioactive solution was diluted with ammonium acetate (1 M, pH = 7.0) to obtain a solution with a volume of 200 µL.

6.1 Concentration-Dependent Radiolabelling Studies with [^{203}Pb]Pb^{2+}

The radiolabelling was performed in an Eppendorf tube with a final volume of 50 µL for each radiolabelling reaction. The ligand was dissolved in Milli-Q water (5 µL, 10^{-2} –10^{-7} M), added to a 0.5 mL Eppendorf tube, and then diluted with ammonium acetate buffer (44 µL, 1 M, pH = 7.0). [^{203}Pb]$PbCl_2$ in hydrochloric acid (312 kBq, 1 µL, 1 M) was added to the ligand solution. As a control experiment, the radiolabelling was performed by using ammonium acetate buffer solution (49 µL, 1 M, pH = 7.0) and [^{203}Pb]$PbCl_2$ in hydrochloric acid (312 kBq, 1 µL, 1 M). The reaction mixture was kept for 5 min at room temperature. An aliquot was spotted on pH paper to ensure a pH of ca. 7 during the radiolabelling reaction. Then, an aliquot was spotted on an iTLC-SG plate and was analyzed via radio-TLC with a mobile phase solution containing EDTA (50 mM, pH = 5.0). Under these TLC conditions, uncomplexed [^{203}Pb]$PbCl_2$ moves with the mobile phase attain an R_f value of 0.8–1.0 (control experiment), whereas the macropa complexes sit closer to the baseline with an R_f value of 0.2. All the radiolabelling reactions were performed in triplicate.

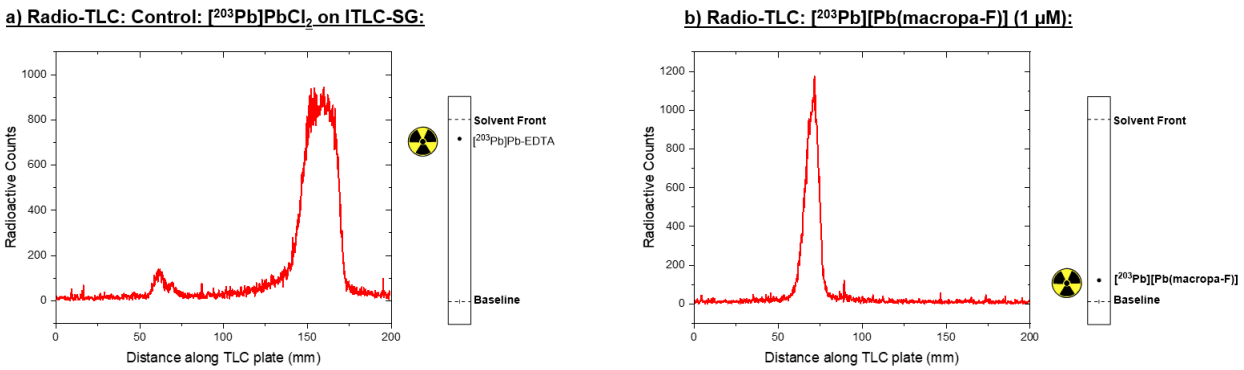


Figure S5: Radio-TLC chromatograms of $[^{203}Pb]Pb^{2+}$ as a) $[^{203}Pb]$Pb-EDTA complex or b) as **$[^{203}Pb][Pb(macropa\text{-}F)]$** complex after spotting the radioactivity on iTLC-SG plates and subsequent development of the TLC plates using radio-TLC (mobile phase: EDTA (50 mM, pH = 5.0)).

Table S4: Concentration-Dependent Radiolabelling Studies of Macropa and Macropa-F with $[^{203}Pb]Pb^{2+}$, as Analyzed by Radio-TLC.

Ligand Concentration	RCY of $[^{203}Pb][Pb(macropa)]$ [%]	RCY of $[^{203}Pb][Pb(macropa\text{-}F)]$ [%]
1 mM	99.8 ± 0.2	99.6 ± 0.3
100 µM	98.2 ± 1.1	99.7 ± 0.3
10 µM	98.8 ± 0.4	99.9 ± 0.1
1 µM	98.7 ± 1.0	99.4 ± 0.7
100 nM	66.6 ± 2.3	27.4 ± 0.7
10 nM	10.7 ± 2.7	5.4 ± 3.1

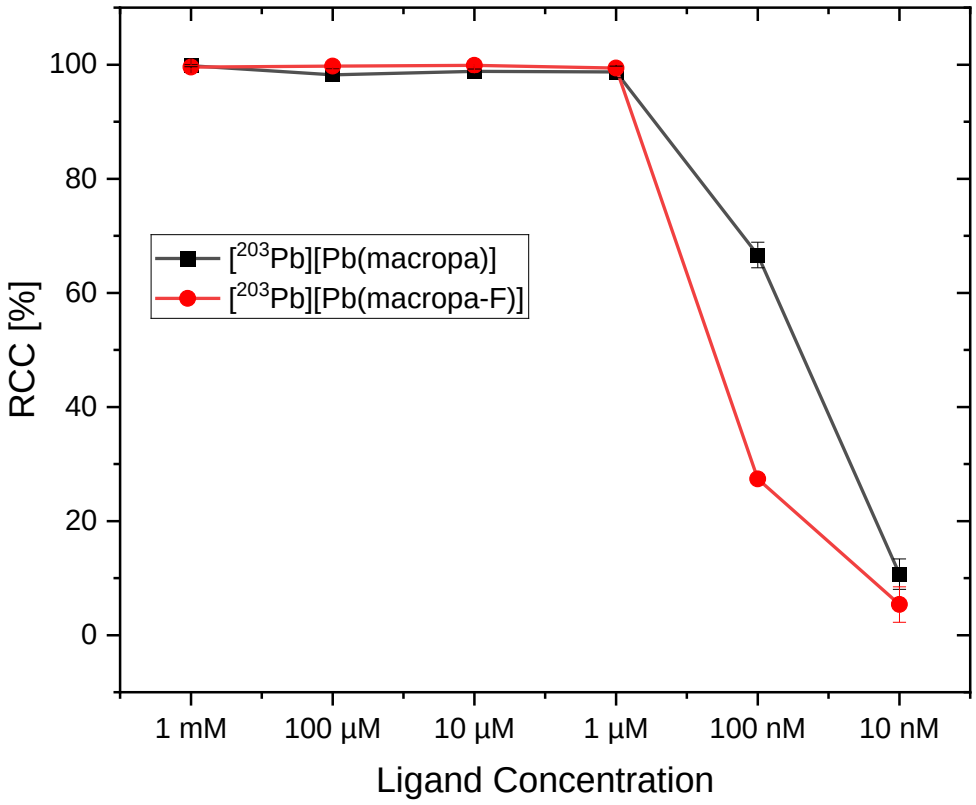


Figure S6: RCCs of [^{203}Pb]Pb^{2+}-labelled **macropa** and **macropa-F** as a function of different chelator concentrations, as evaluated with radio-TLC (conditions: radiolabelling in ammonium acetate buffer solution (1 M, pH = 7.0), rt, 5 min).

6.2 HPLC chromatograms

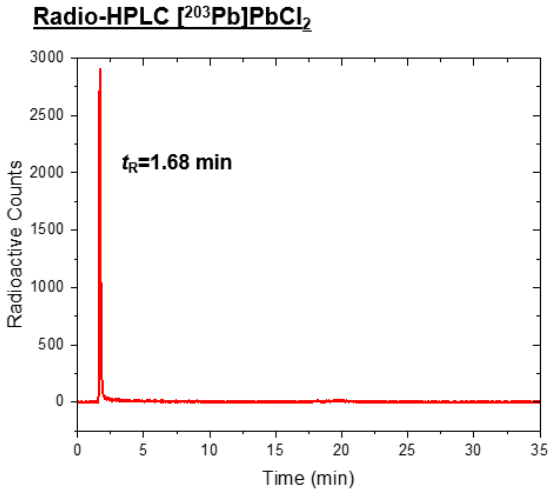


Figure S7: Radio-HPLC chromatogram of [^{203}Pb]$PbCl_2$ (HPLC method: *system 3*).

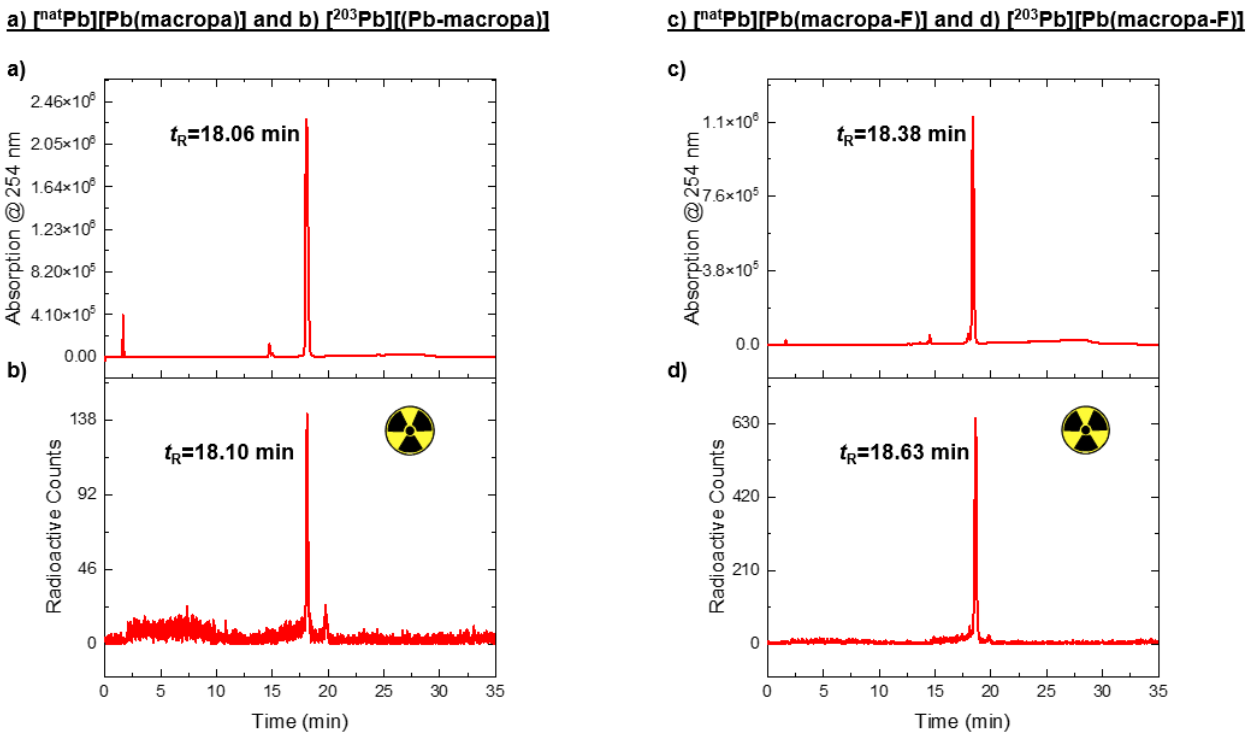


Figure S8: Radio-HPLC chromatograms of a) **[^{nat}Pb][Pb(macropa)]**, b) **[^{203}Pb][Pb(macropa)]**, c) **[^{nat}Pb][Pb(macropa-F)]**, and d) **[^{203}Pb][Pb(macropa-F)]** (HPLC method: *system 3*).

6.3 Human Serum Stability Studies of [^{203}Pb][Pb(macropa)] and [^{203}Pb][Pb(macropa-F)]

Human serum stability studies were performed as previously reported with minor modifications.[9] The ^{203}Pb-radiolabelled complex was initially formed in a 1.5-mL Eppendorf tube with a final volume of 380 µL for each radiolabelling reaction. The ligand (20 µL, 10^{-4} M, final ligand

concentration: 5 µM) was added to the Eppendorf and was diluted with ammonium acetate buffer solution (160 µL, 1 M, pH = 7.0). Then, [^{203}Pb]$PbCl_2$ in ammonium acetate buffer (6.66 MBq, 20 µL, 1 M, pH = 7.0) was added to the ligand solution. The reaction mixture was kept for 10 min at room temperature. An aliquot (10 µL) was spotted on an iTLC-SG plate and was analyzed via radio-TLC (mobile phase: EDTA (50 mM, pH = 5.0)). In a control experiment, the serum stability study was performed in a similar procedure by using ammonium acetate buffer solution (180 µL, 1 M, pH = 7.0), [^{203}Pb]$PbCl_2$ in ammonium acetate buffer (6.66 MBq, 20 µL, 1 M, pH = 7.0) and human serum (190 µL). After analyzing the TLC plates and ensuring quantitative radiolabelling (0 h), human serum (190 µL) was added to the radioactive solution containing [^{203}Pb][Pb(macropa)] complexes. The Eppendorf tubes containing [^{203}Pb][Pb(macropa)] complexes were incubated at 37 °C, and the stability of the intact complexes was evaluated after several time points via radio-TLC. The experiments were performed in triplicate.

Table S5: Human Serum Stability Studies of [^{203}Pb][Pb(macropa)] and [^{203}Pb][Pb(macropa-F)] (Experiments were Performed in Triplicate).

	[^{203}Pb][Pb(macropa)]	**[^{203}Pb][Pb(macropa-F)]**
Time	Intact Complex [%]	Intact Complex [%]
0 h	99.9	99.7 ± 0.2
1 h	99.9	99.5 ± 0.4
24 h	99.7 ± 0.3	99.3 ± 0.5
48 h	99.1 ± 0.6	99.4 ± 0.4
72 h	98.5 ± 1.8	99.4 ± 0.5
96 h	99.6 ± 0.2	99.5 ± 0.2
120 h	99.4 ± 0.7	99.0 ± 0.8

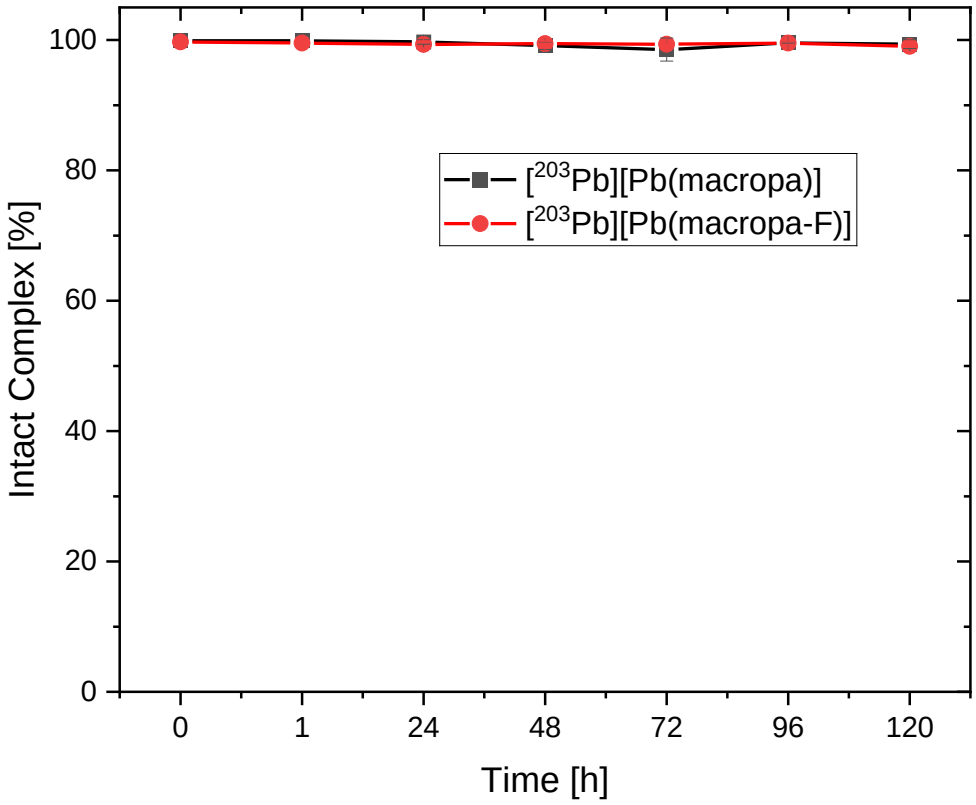


Figure S9: Human serum stability studies of **[^{203}Pb][Pb(macropa)]** and **[^{203}Pb][Pb(macropa-F)]** over time, as analyzed via radio-TLC.

7. Radiolabelling of Macropa and Macropa-F with [^{207}Bi]Bi^{3+}

Radioactive bismuth-207 was purchased as [^{207}Bi]Bi(NO_3)$_3$ in nitric acid (4 N) from Los Alamos National Laboratory, NM, USA and its Isotope Production and Application Program stock supply. The purity of [^{207}Bi]Bi(NO_3)$_3$ was verified by ICP-OES as >99%. The initial activity of the [^{207}Bi]Bi(NO_3)$_3$ was 10 µCi (370 kBq). An aliquot of the radioactivity was diluted with MES buffer (0.5 M, pH = 5.53) for the concentration-dependent radiolabelling studies and the human serum stability studies. The detection of the radioactivity was performed by using a gamma counter, detecting the 570 keV gamma emission of bismuth-207. In Figure S10, the detailed workflow for the quantification and detection of bismuth-207 via a gamma counter is illustrated.

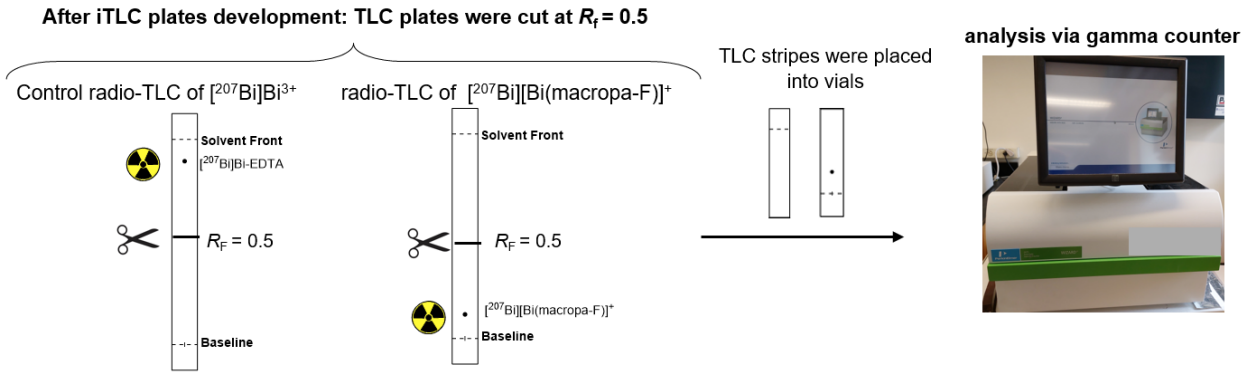


Figure S10: Quantification process of bismuth-207 from iTLC plates via gamma counter.

7.1 Concentration-Dependent Radiolabelling Studies with $[^{207}Bi]Bi^{3+}$

Radiolabelling reactions were carried out in Eppendorf tubes in a final volume of 100 µL. The ligand (10 µL, 10^{-2} –10^{-7} M) in MES buffer (0.5 M, pH = 5.53) was added to the Eppendorf tube and was diluted with additional MES buffer (86 µL, 0.5 M, pH = 5.53). Then, $[^{207}Bi]Bi(NO_3)_3$ in MES buffer (4 µL, 380 Bq) was added to the ligand solution. As a control experiment, the radiolabelling was performed using MES buffer solution (96 µL, 0.5 M, pH = 5.53) and $[^{203}Bi]BiNO_3$ in MES buffer (380 Bq, 4 µL). The reaction mixture was incubated for 8 min at room temperature. An aliquot (1 µL) was spotted on pH paper to ensure a pH of ca. pH = 5–6 during radiolabelling. Then, an aliquot (10 µL) was spotted on an iTLC-SG plate and was analyzed via the gamma counter as described above (Figure S10). The mobile phase for the TLC was a solution containing EDTA (50 mM, pH = 5.50). Under these TLC conditions, $[^{207}Bi]Bi(NO_3)_3$ as a EDTA complex moved with the mobile phase (control experiment), attaining an R_f between 0.8–1.0, and the corresponding macropa complexes remained near the baseline (R_f between 0.1–0.5). All the radiolabelling reactions were performed in triplicate.

Table S6: Concentration-Dependent Radiolabelling Studies of Macropa and Macropa-F with $[^{207}Bi]Bi^{3+}$, as Analyzed by Radio-TLC.

Ligand Concentration	RCY of $[^{207}Bi][Bi(macropa)]^+$ [%]	RCY of $[^{207}Bi][Bi(macropa\text{-}F)]^+$ [%]
1 mM	98.9 ± 0.8	99.3 ± 0.5
100 µM	98.9 ± 0.9	98.7 ± 0.2
10 µM	96.2 ± 1.6	98.3 ± 0.6
1 µM	94.0 ± 2.3	96.3 ± 1.4
100 nM	37.3 ± 4.3	53.4 ± 7.3
10 nM	5.6 ± 0.4	11.3 ± 4.5

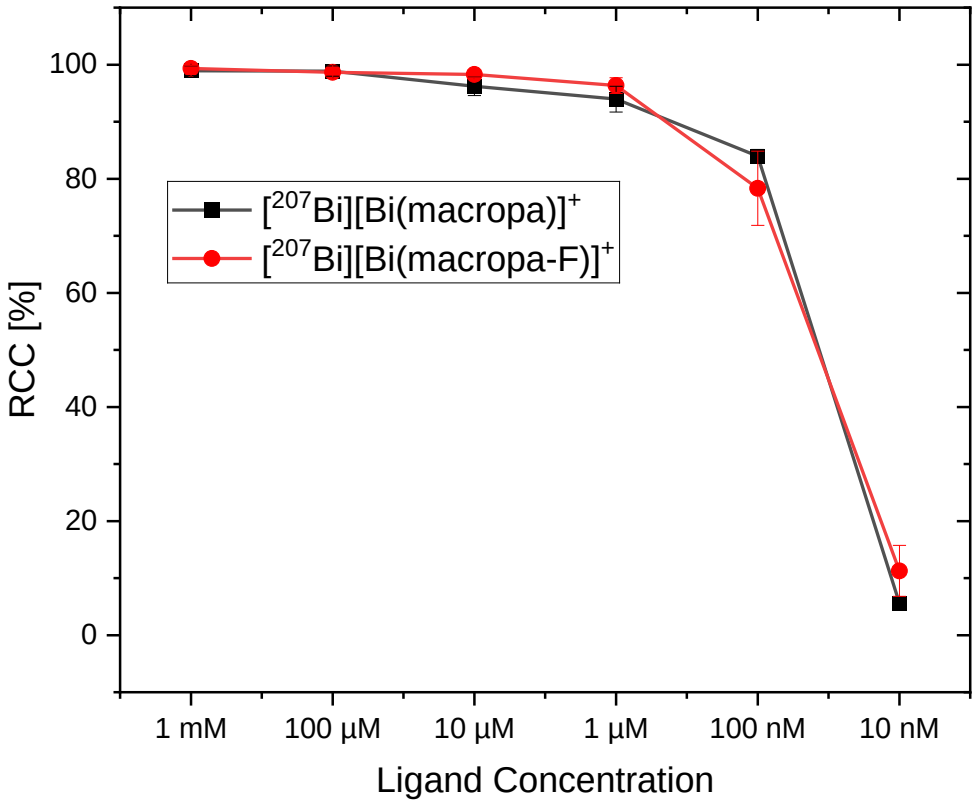


Figure S11: Radiolabelling of **macropa** and **macropa-F** with [^{207}Bi]Bi^{3+} using different ligand concentration, as evaluated by radio-TLC (conditions: radiolabelling in MES buffer solution (0.5 M, pH = 5.53), rt, 8 min).

7.2 Human Serum Stability Studies of [^{207}Bi][Bi(macropa)]$^{+}$ and [^{207}Bi][Bi(macropa-F)]$^{+}$

Human serum stability studies were performed as previously reported with minor modifications.[9] ^{207}Bi radiolabelling for these studies was carried out in an Eppendorf tube with a final volume of 380 µL. The ligand (20 µL, 10^{-4} M, final ligand concentration: 5 µM) was added to the Eppendorf tube and was diluted with MES buffer solution (114 µL, 0.5 M, pH = 5.53) and PBS (50 µL, pH = 7.4). Then, [^{207}Bi]Bi$(NO_3)_3$ in MES buffer (1.60 kBq, 16 µL, 0.5 M, pH = 5.53) was added to the ligand solution. The reaction mixture was incubated for 10 min at room temperature. An aliquot (10 µL) was spotted on an iTLC-SG plate and was analyzed via radio-TLC (mobile phase: EDTA (50 mM, pH = 5.50)). In a control experiment, the serum stability study was performed in a similar procedure by using MES buffer solution (134 µL, 0.5 M, pH = 5.53), PBS (50 µL, pH = 7.4), [^{207}Bi]Bi$(NO_3)_3$ in MES buffer (1.60 kBq, 16 µL, 0.5 M, pH = 5.53) and human serum (190 µL).

After analyzing the TLC plates and ensuring quantitative radiolabelling (0 h), human serum (190 µL) was added to the radioactive solution containing [^{207}Bi][Bi(macropa)]$^{+}$ complexes. The Eppendorf tubes containing [^{207}Bi][Bi(macropa)]$^{+}$ complexes were incubated at 37 °C, and the stability of the intact complexes was evaluated via the gamma counter after several time points via radio-TLC. The experiments were performed in triplicate.

Table S7: Human Serum Stability of [^{207}Bi][Bi(macropa)]$^{+}$ and [^{207}Bi][Bi(macropa-F)]$^{+}$ (Experiments were Performed in Triplicate).

	[^{207}Bi][Bi(macropa)]$^{+}$	**[^{207}Bi][Bi(macropa-F)]$^{+}$**
Time	**Intact Complex [%]**	**Intact Complex [%]**
0 h	99.6 ± 0.2	99.0 ± 0.5
1 h	98.5 ± 1.2	97.9 ± 0.9
3 h	98.3 ± 0.5	99.2 ± 0.4
24 h	99.2 ± 0.6	98.6 ± 0.6
48 h	99.9	99.9
72 h	99.8 ± 0.1	98.5 ± 0.3
96 h	99.7 ± 0.4	99.5 ± 0.3
120 h	99.5 ± 0.1	99.5 ± 0.5

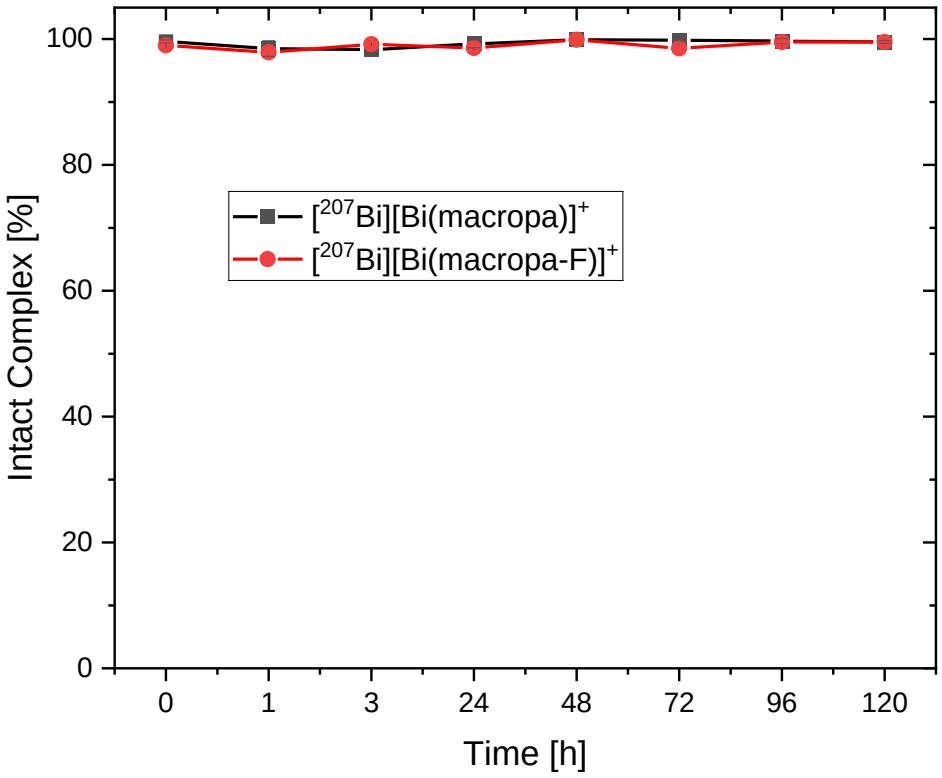


Figure S12: Human serum stability of **[^{207}Bi][Bi(macropa)]$^{+}$** and **[^{207}Bi][Bi(macropa-F)]$^{+}$** over time.

8. Distribution Coefficient Determination

The distribution coefficients of the radiometal complexes were determined in biphasic mixture of PBS (pH = 7.4) and *n*-octanol using the shake-flask method.[10] The radiometal complexes (lead-207 metal complexes in ammonium acetate (pH = 7.00; 10 µL) and the bismuth-207 complexes in MES buffer (pH = 5.53; 40 µL)) were added to an Eppendorf tube containing PBS (490 µL for both lead-203 complexes; 460 µL for both bismuth-207 complexes) and then *n*-octanol (500 µL) was added. The mixture was vigorously shaken for 5 min at 25 °C. The organic and aqueous phases were separated via centrifugation (3 min, 4000 rpm). Subsequently, aliquots (250 µL) from each phase were measured by gamma counting. The log *D* value at pH = 7.4 was calculated by using the following equation:

$$-\log D_{pH=7.4} = \log \frac{A_{oct}}{A_{PBS}} \quad (1)$$

where A_{oct} describes the decay corrected counts per min from the *n*-octanol phase, the A_{PBS} is the decay corrected counts per min from the aqueous PBS phase.

The reported value is the average of 4 measurements:

$$\log D_{pH=7.4}\ (\mathbf{[^{203}Pb][Pb(macropa)]}) = -0.78 \pm 0.04\ (n = 4)$$

$$\log D_{pH=7.4}\ (\mathbf{[^{203}Pb][Pb(macropa\text{-}F)]}) = -0.37 \pm 0.01\ (n = 4)$$

$$\log D_{pH=7.4}\ (\mathbf{[^{207}Bi][Bi(macropa)]^{+}}) = -2.09 \pm 0.12\ (n = 4)$$

$$\log D_{pH=7.4}\ (\mathbf{[^{207}Bi][Bi(macropa\text{-}F)]^{+}}) = -1.83 \pm 0.24\ (n = 4)$$

9. Spectra

9.1 NMR Spectra

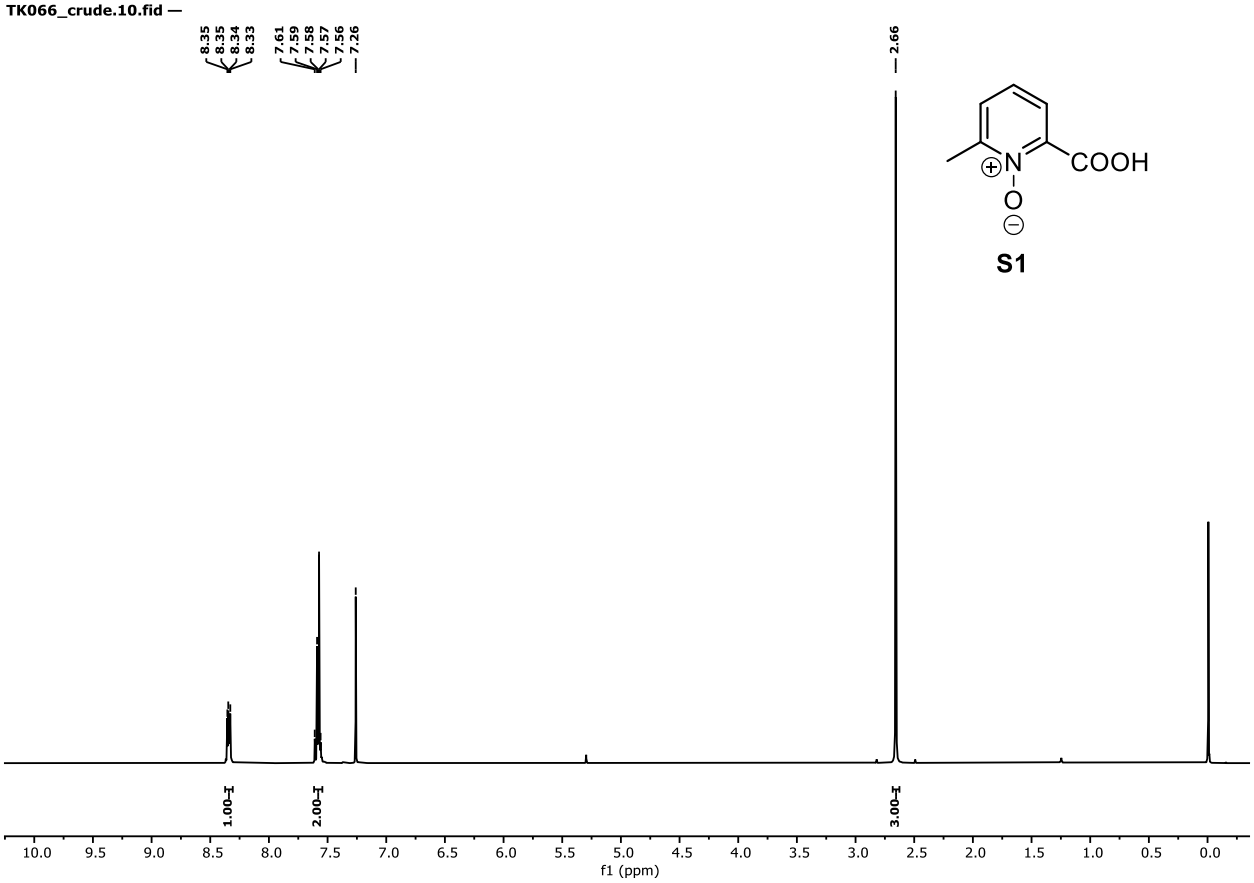


Figure S13: ^{1}H NMR spectrum of **S1** in $CDCl_3$ (400 MHz, 298 K).

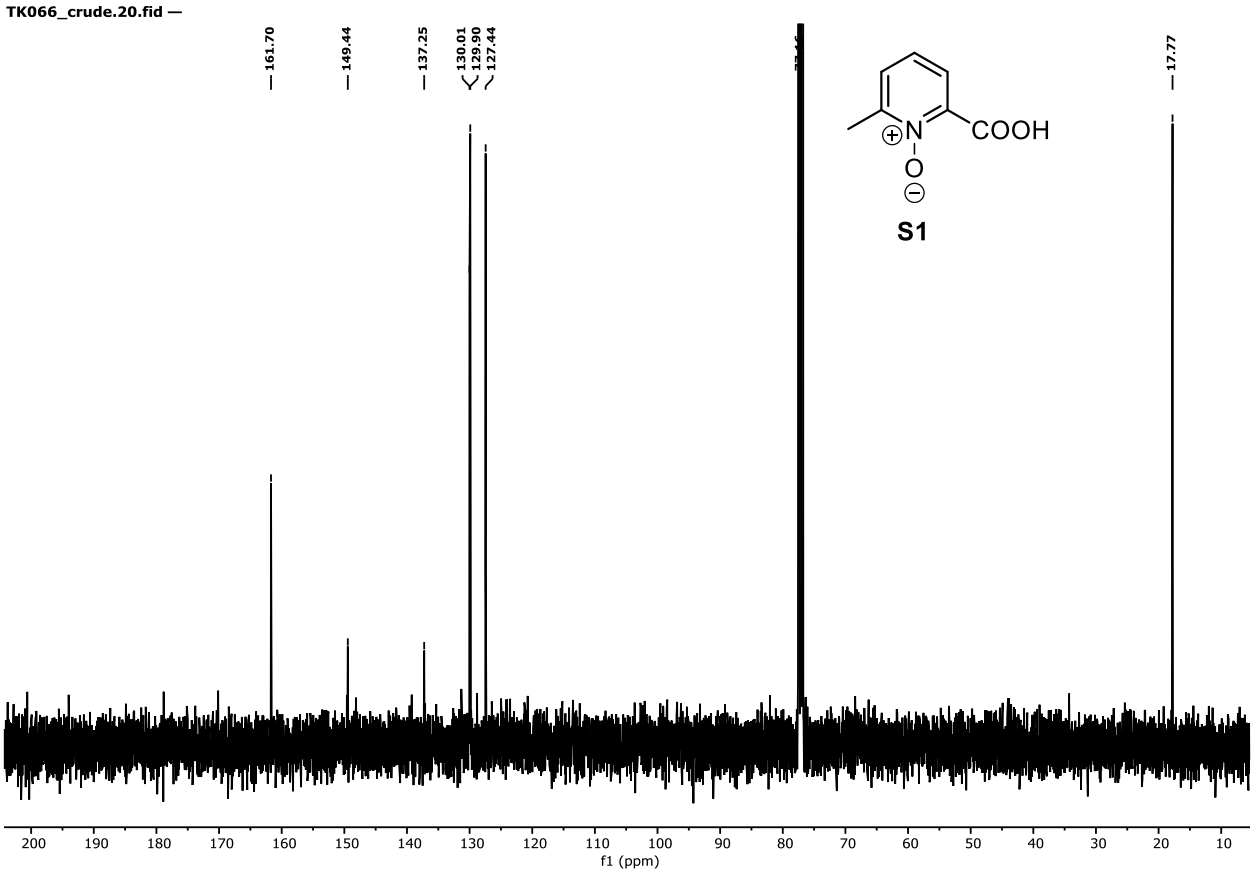


Figure S14: $^{13}C\{^{1}H\}$ NMR spectrum of **S1** in $CDCl_3$ (101 MHz, 298 K).

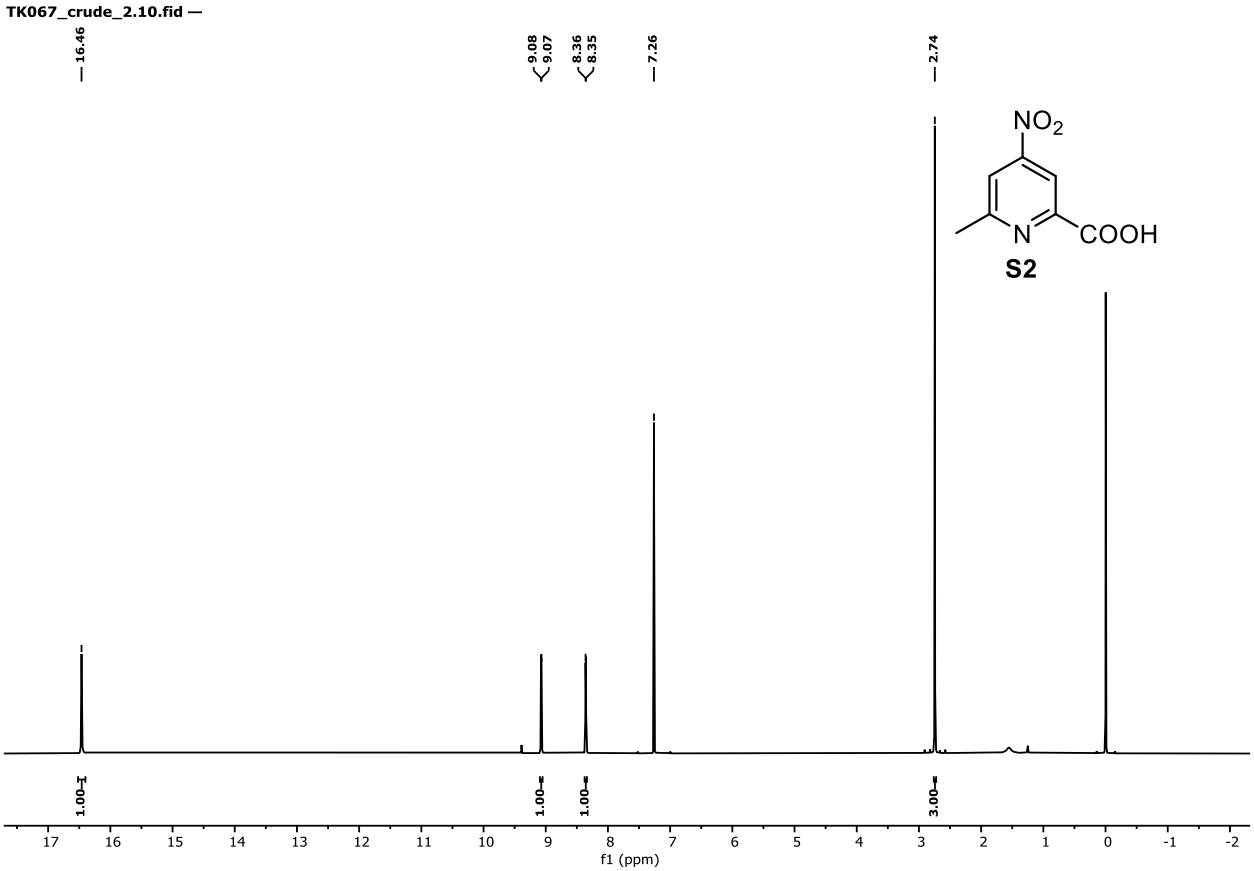


Figure S15: ^{1}H NMR spectrum of **S2** in $CDCl_3$ (400 MHz, 298 K).

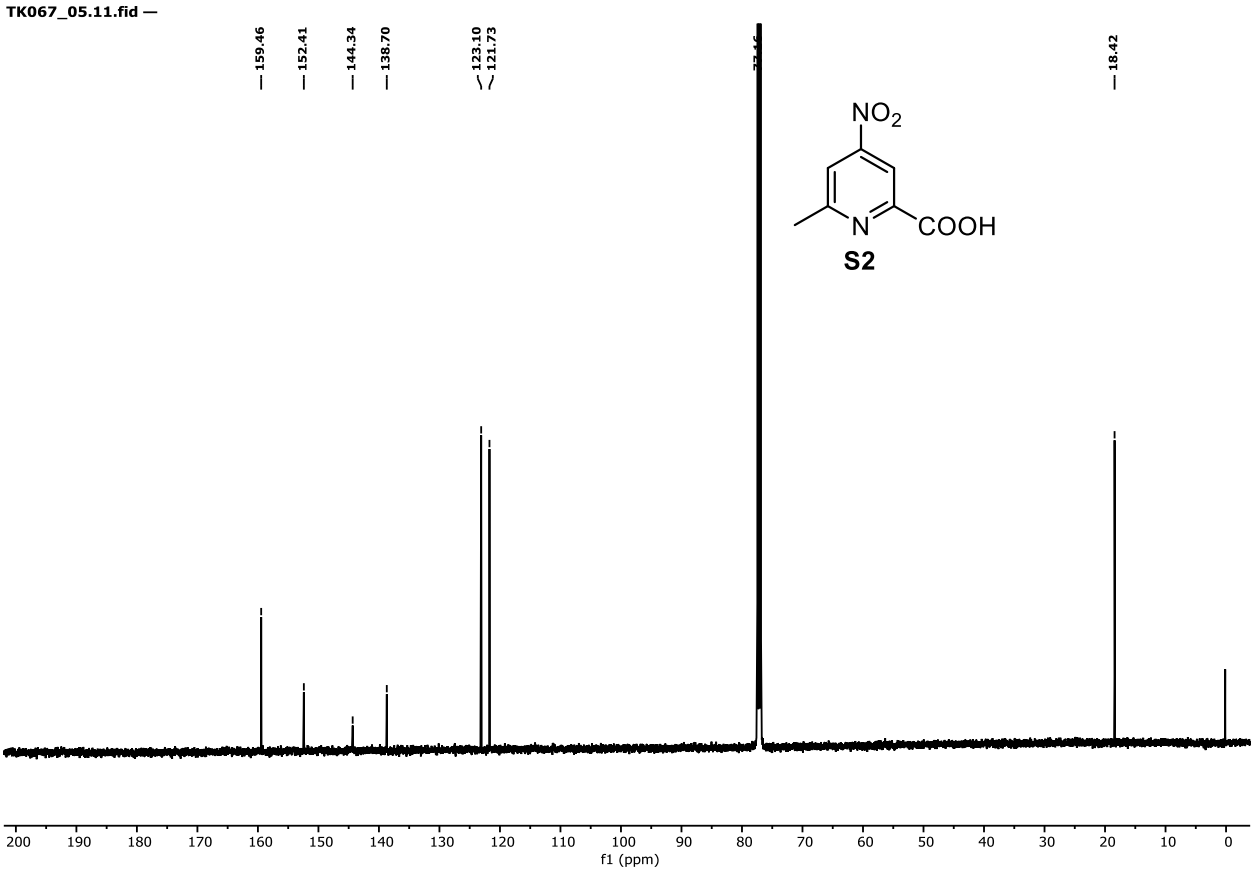


Figure S16: $^{13}C\{^{1}H\}$ NMR spectrum of **S2** in $CDCl_3$ (126 MHz, 298 K).

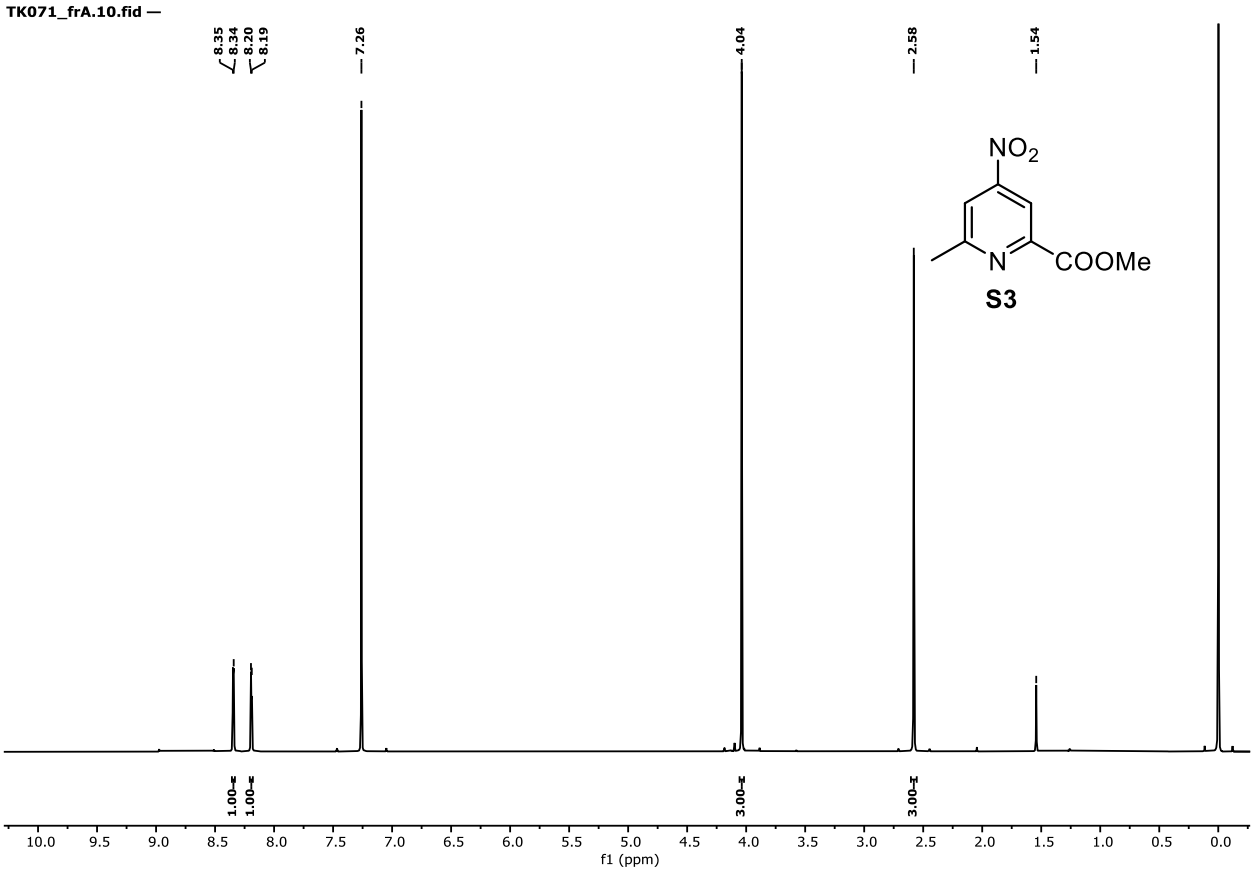


Figure S17: ^{1}H NMR spectrum of **S3** in $CDCl_3$ (500 MHz, 298 K).

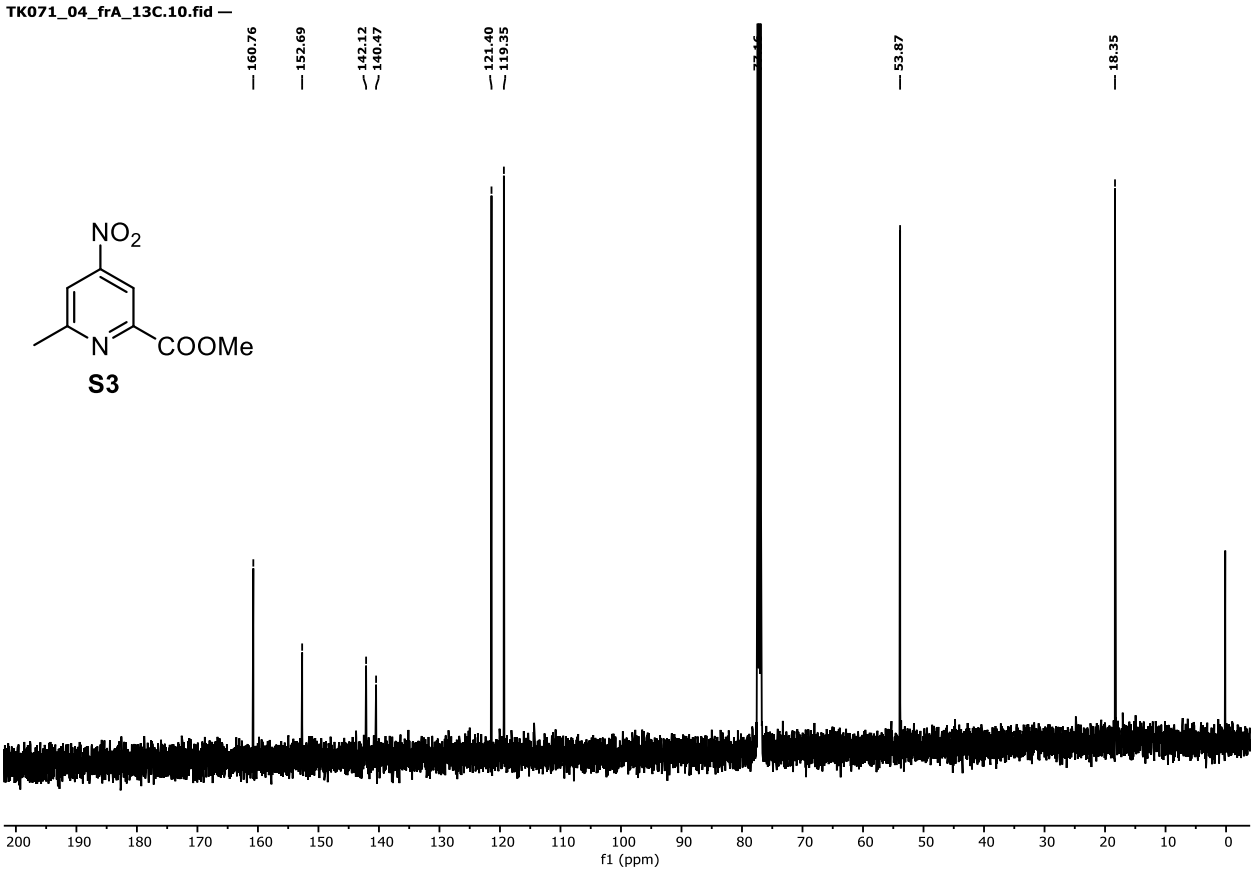


Figure S18: $^{13}C\{^{1}H\}$ NMR spectrum of **S3** in $CDCl_3$ (126 MHz, 298 K).

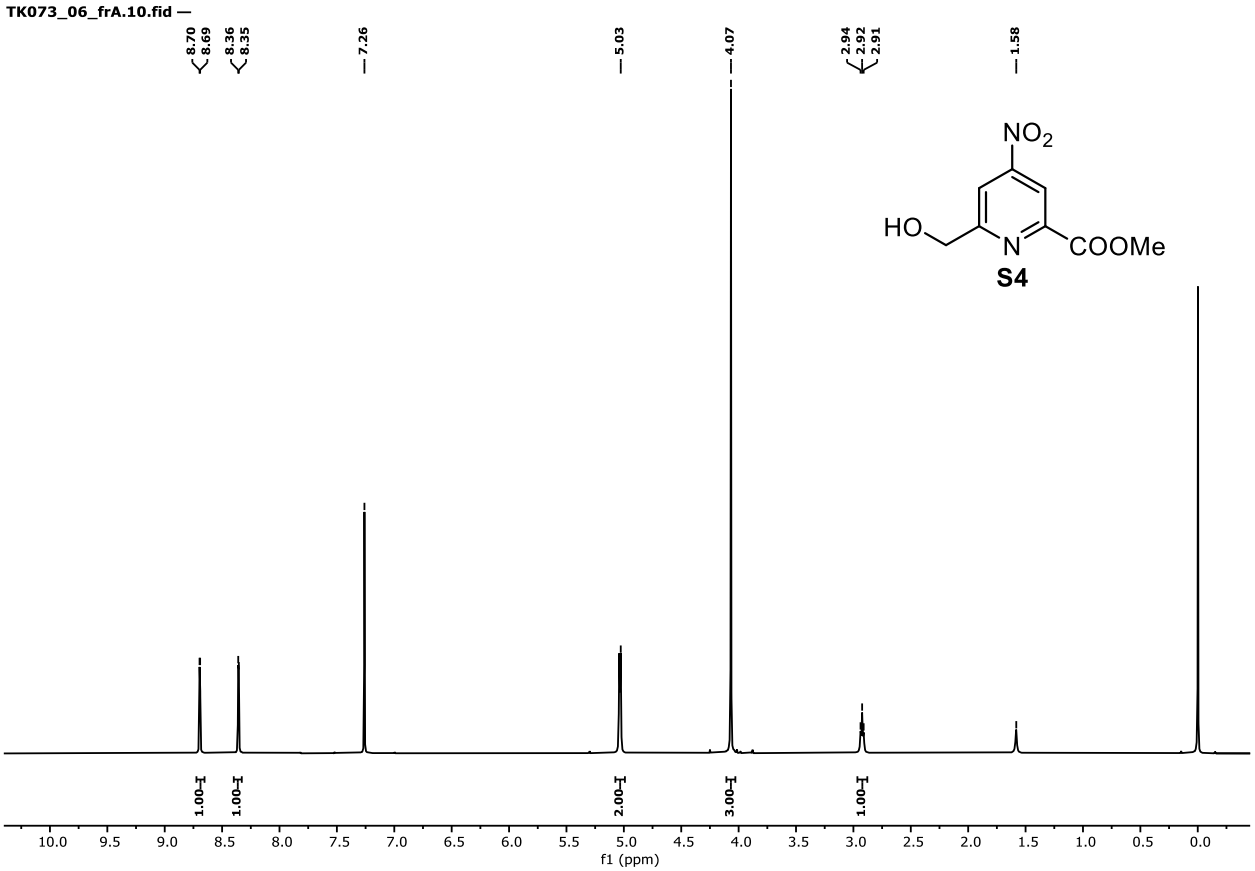


Figure S19: ^{1}H NMR spectrum of **S4** in $CDCl_3$ (400 MHz, 298 K).

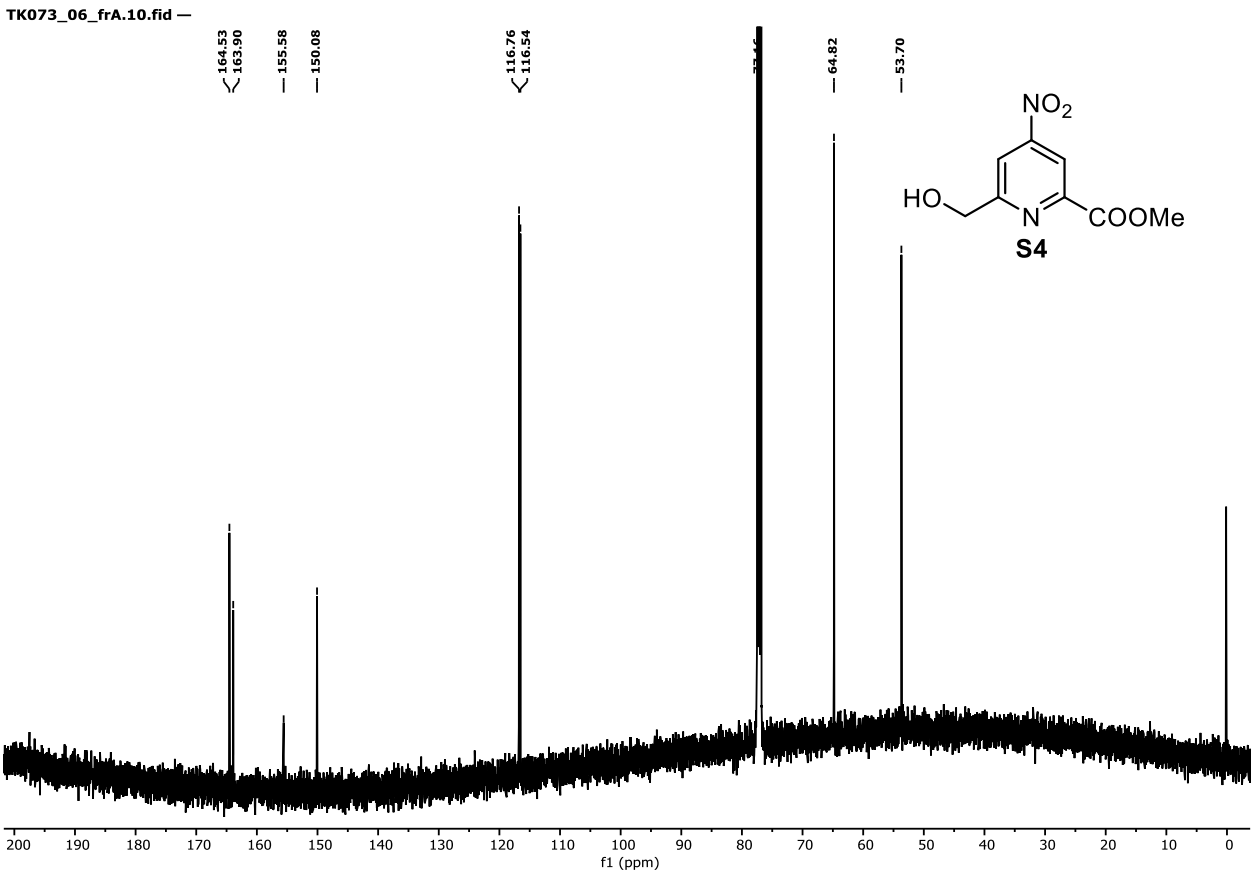


Figure S20: $^{13}C\{^{1}H\}$ NMR spectrum of **S4** in $CDCl_3$ (126 MHz, 298 K).

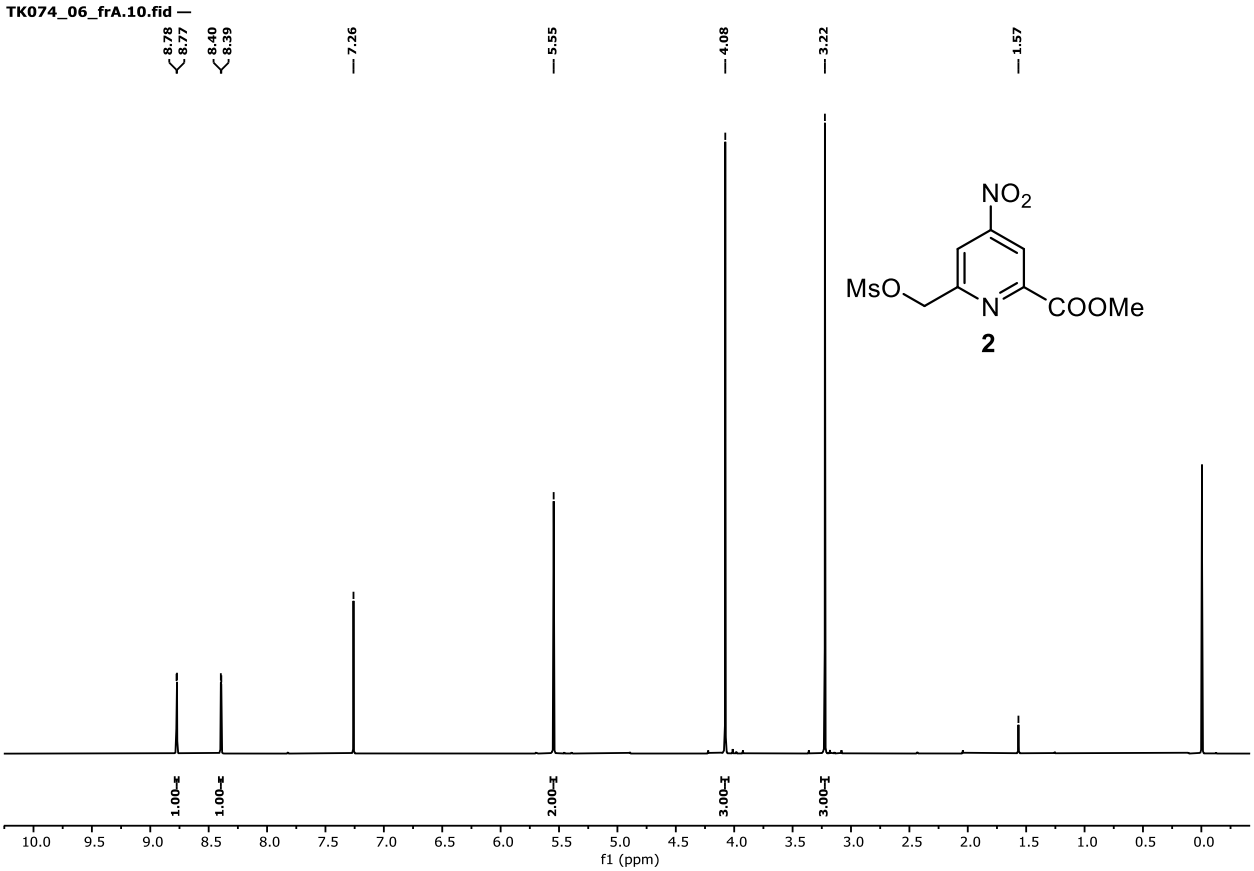


Figure S21: ^{1}H NMR spectrum of **2** in $CDCl_3$ (500 MHz, 298 K).

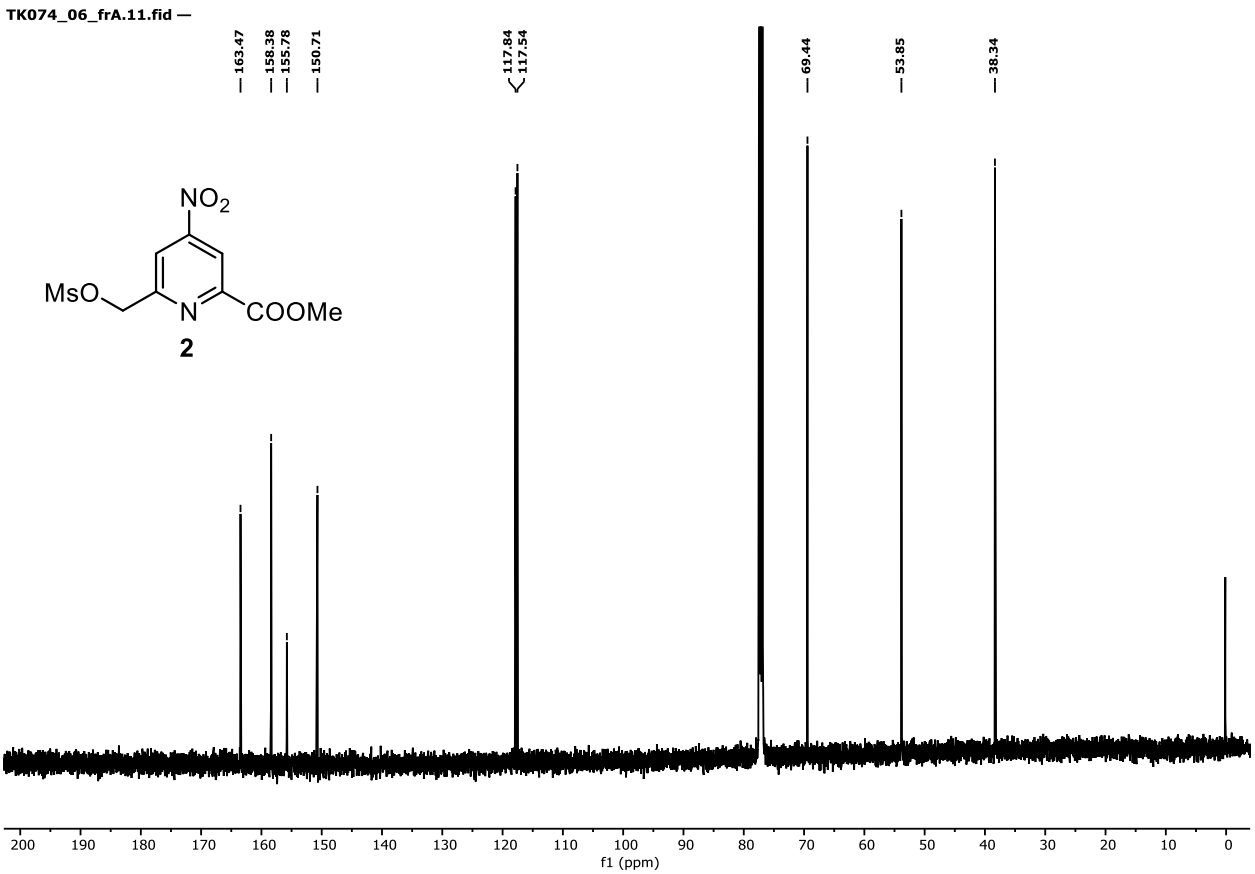


Figure S22: $^{13}C\{^{1}H\}$ NMR spectrum of **2** in $CDCl_3$ (126 MHz, 298 K).

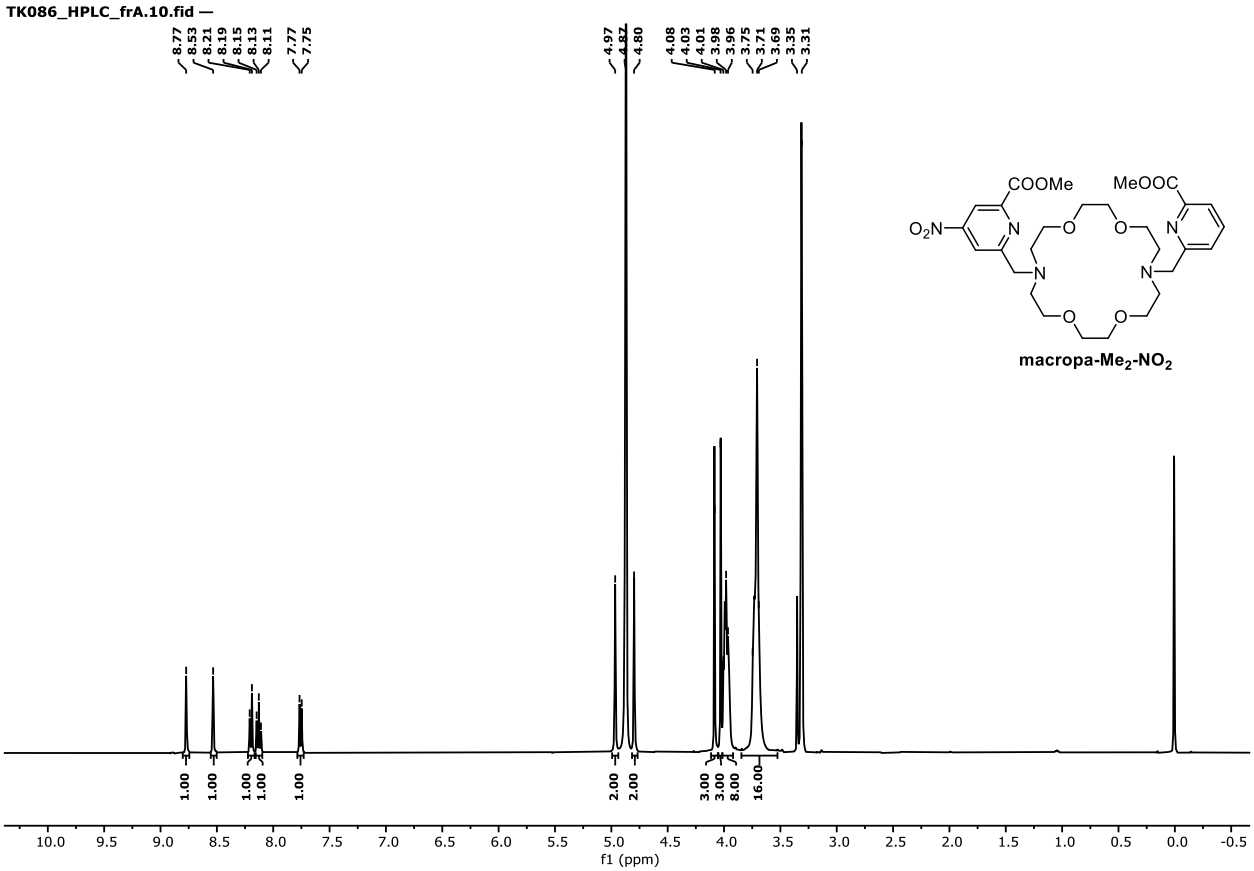


Figure S23: ^{1}H NMR spectrum of **macropa-Me$_2$-NO$_2$** in CD_3OD (400 MHz, 298 K).

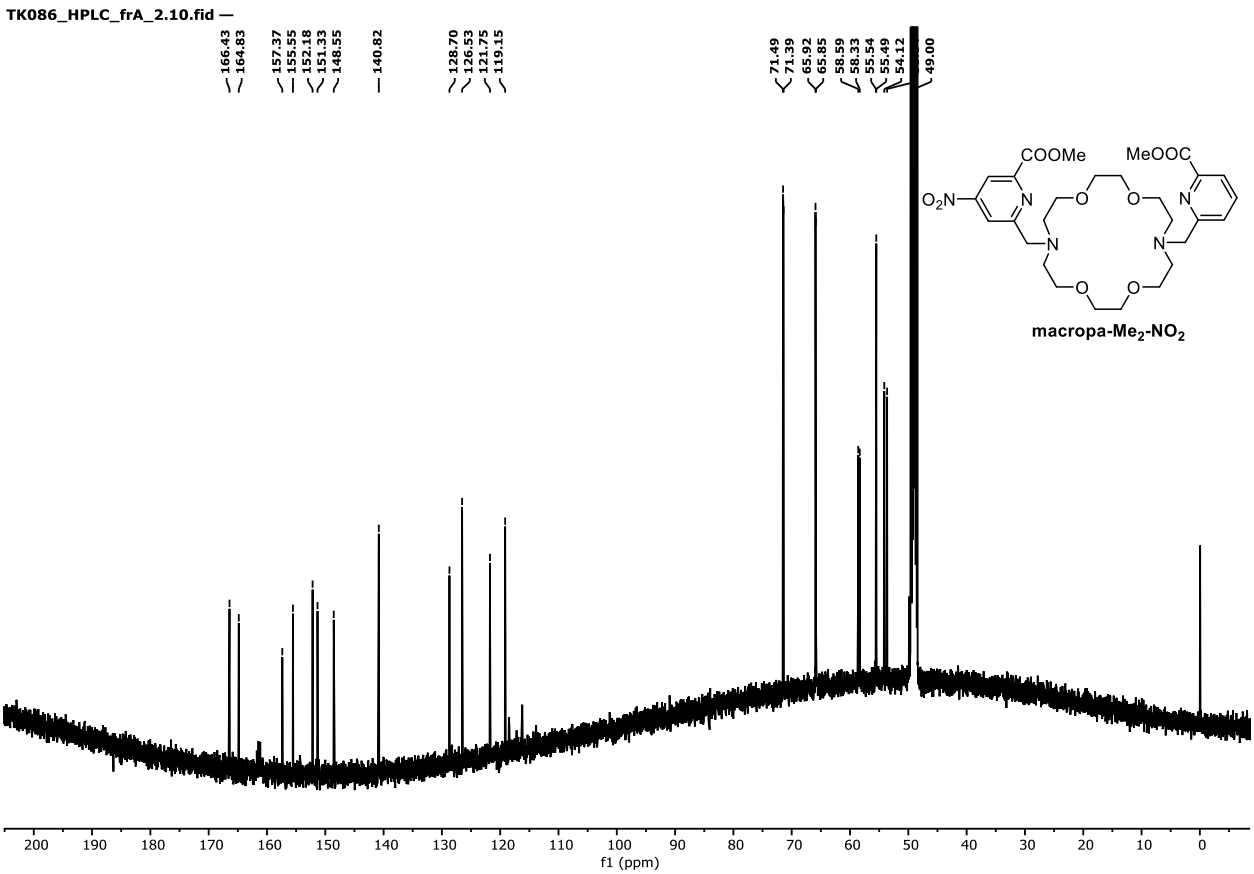


Figure S24: $^{13}C\{^{1}H\}$ NMR spectrum of **macropa-Me$_2$-NO$_2$** in CD_3OD (126 MHz, 298 K).

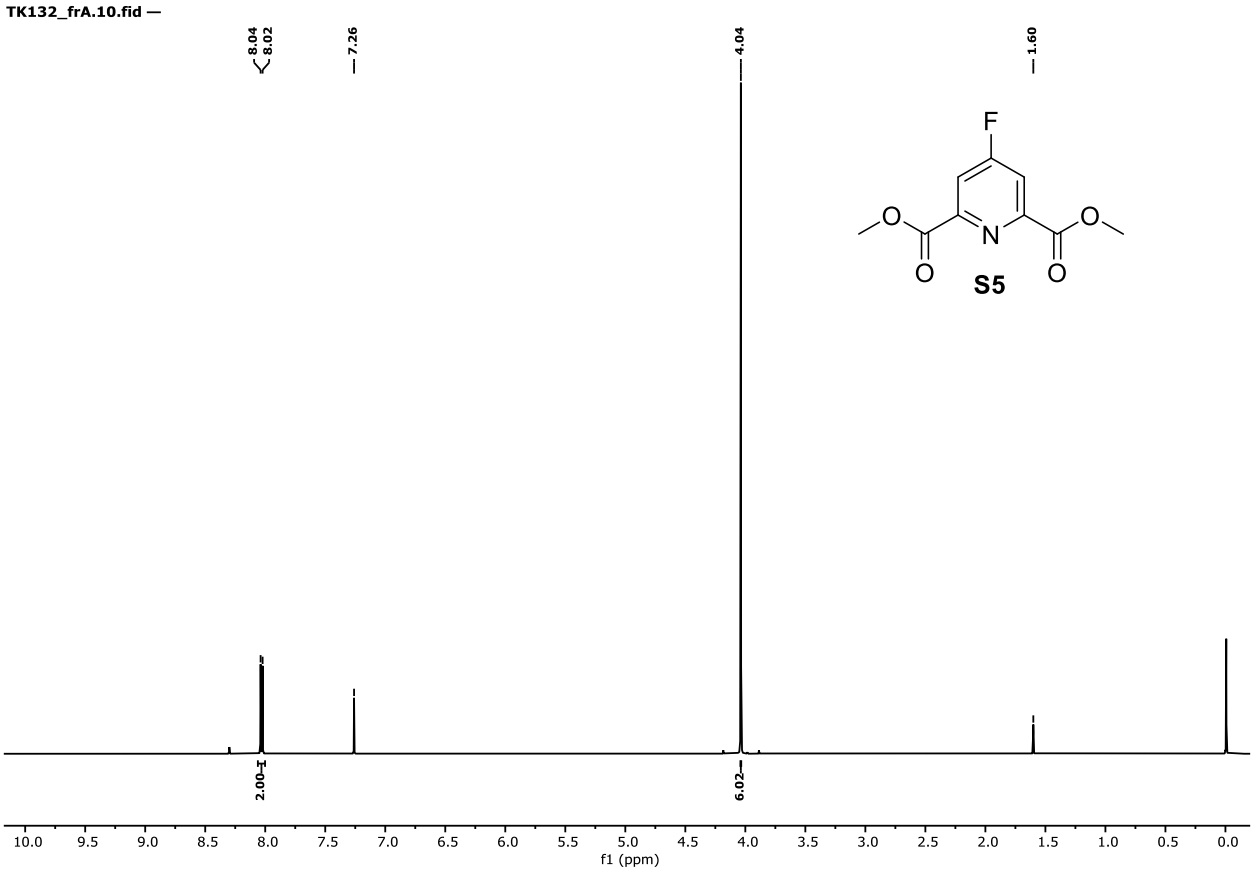


Figure S25: ^{1}H NMR spectrum of **S5** in $CDCl_3$ (500 MHz, 298 K).

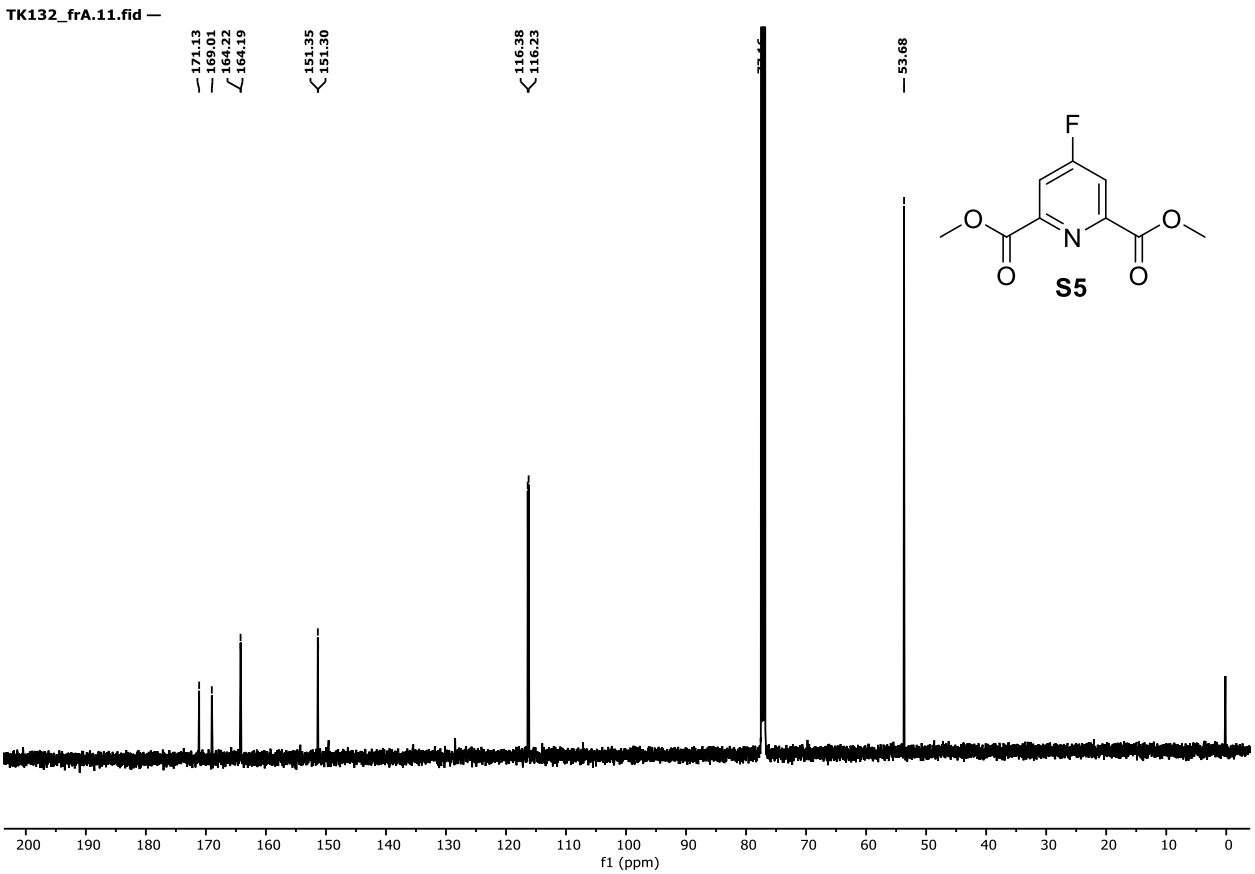


Figure S26: $^{13}C\{^{1}H\}$ NMR spectrum of **S5** in $CDCl_3$ (126 MHz, 298 K).

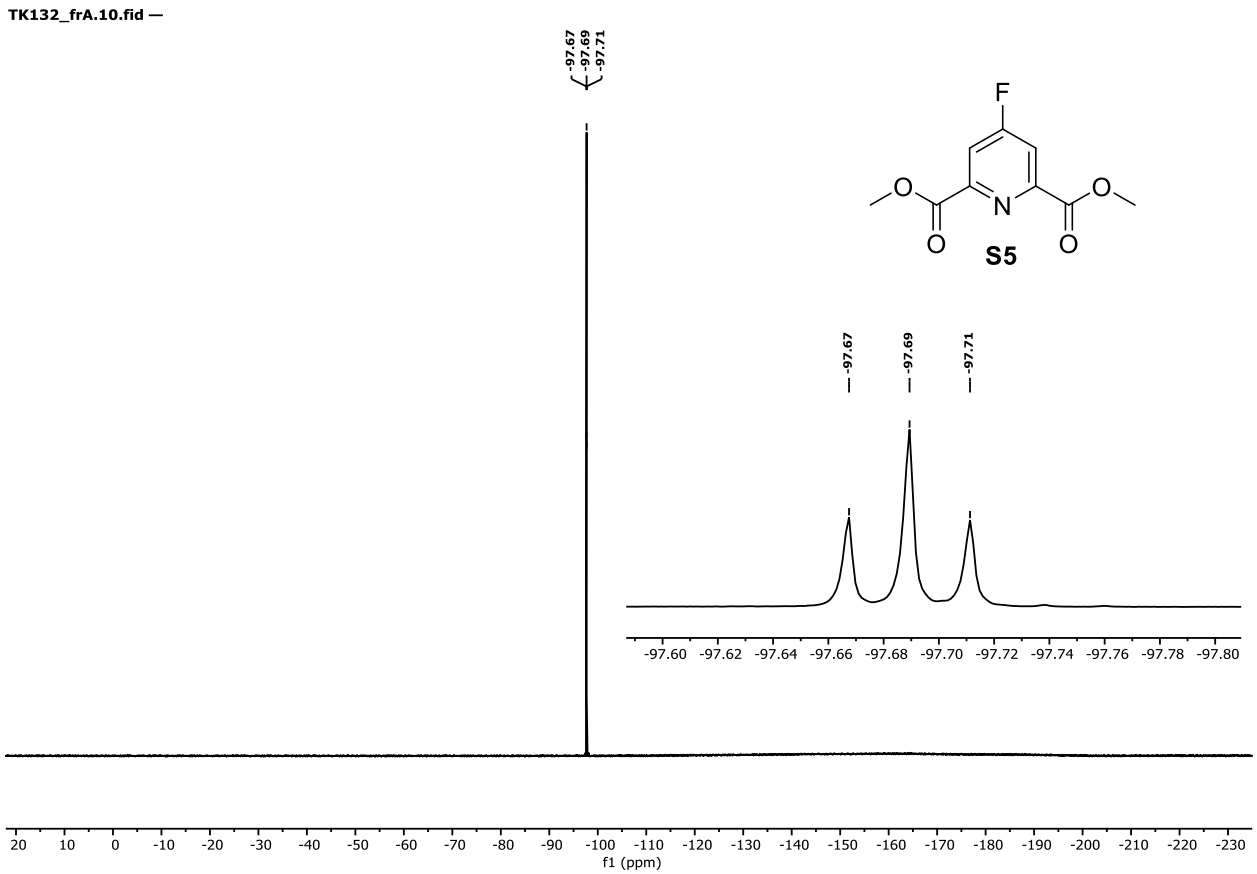


Figure S27: ^{19}F NMR spectrum of **S5** in $CDCl_3$ (376 MHz, 298 K).

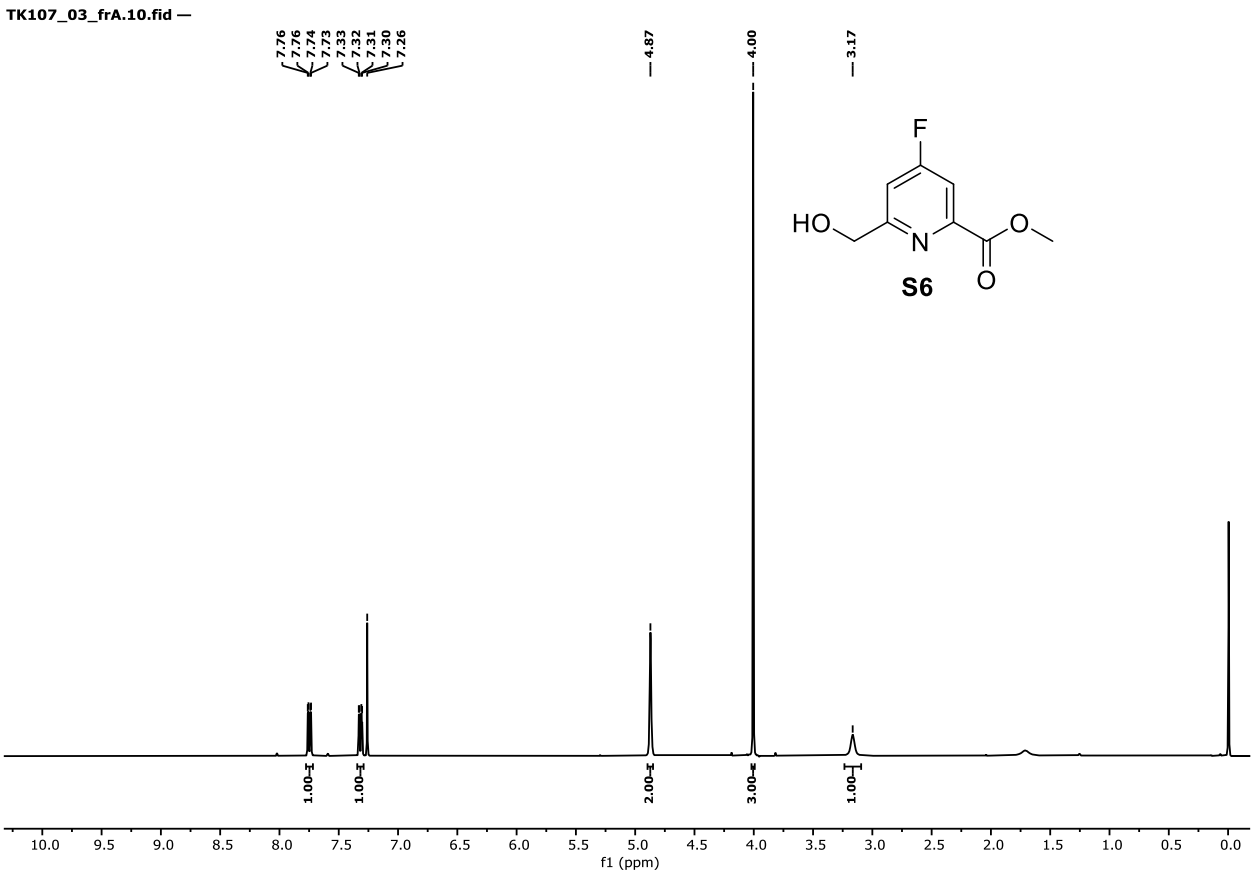


Figure S28: ^{1}H NMR spectrum of **S6** in $CDCl_3$ (400 MHz, 298 K).

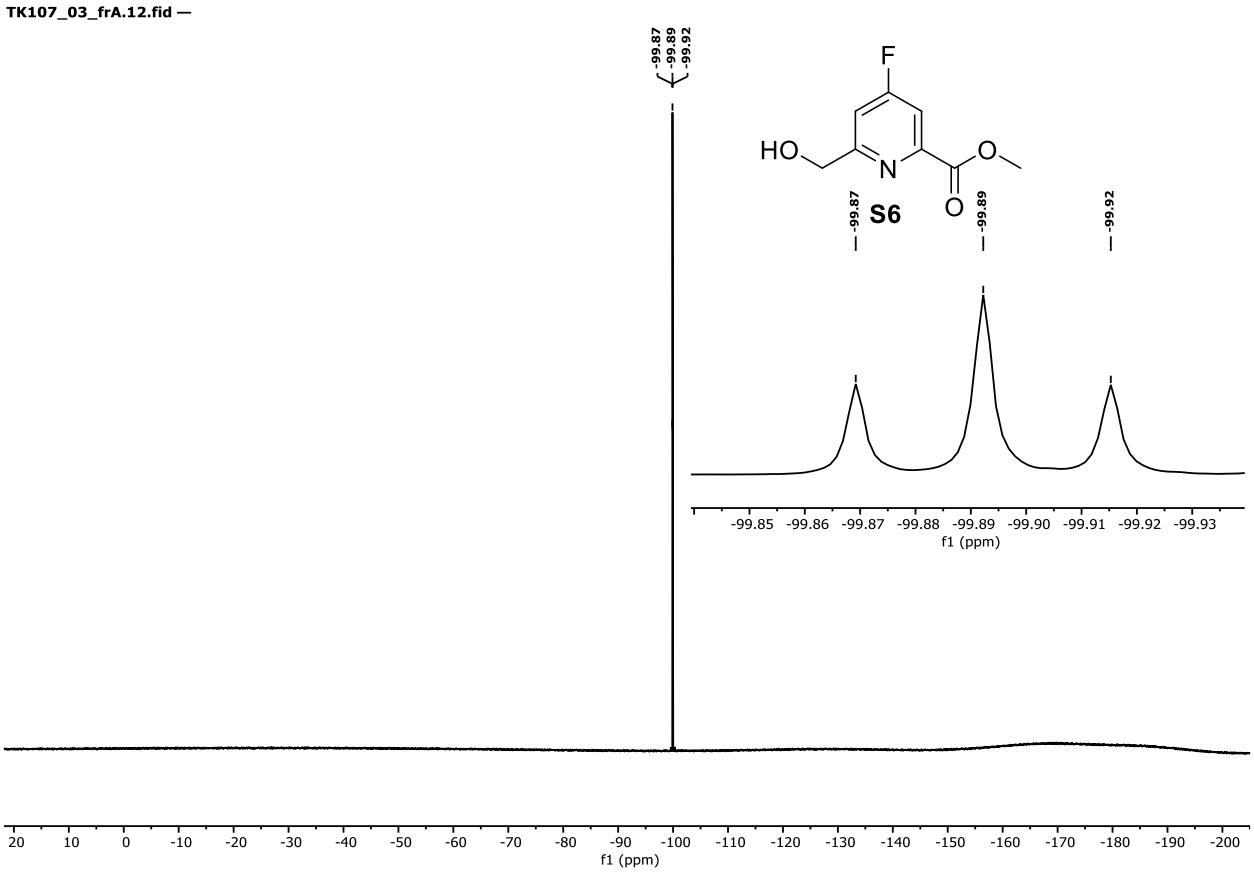


Figure S29: ^{19}F NMR spectrum of **S6** in $CDCl_3$ (376 MHz, 298 K).

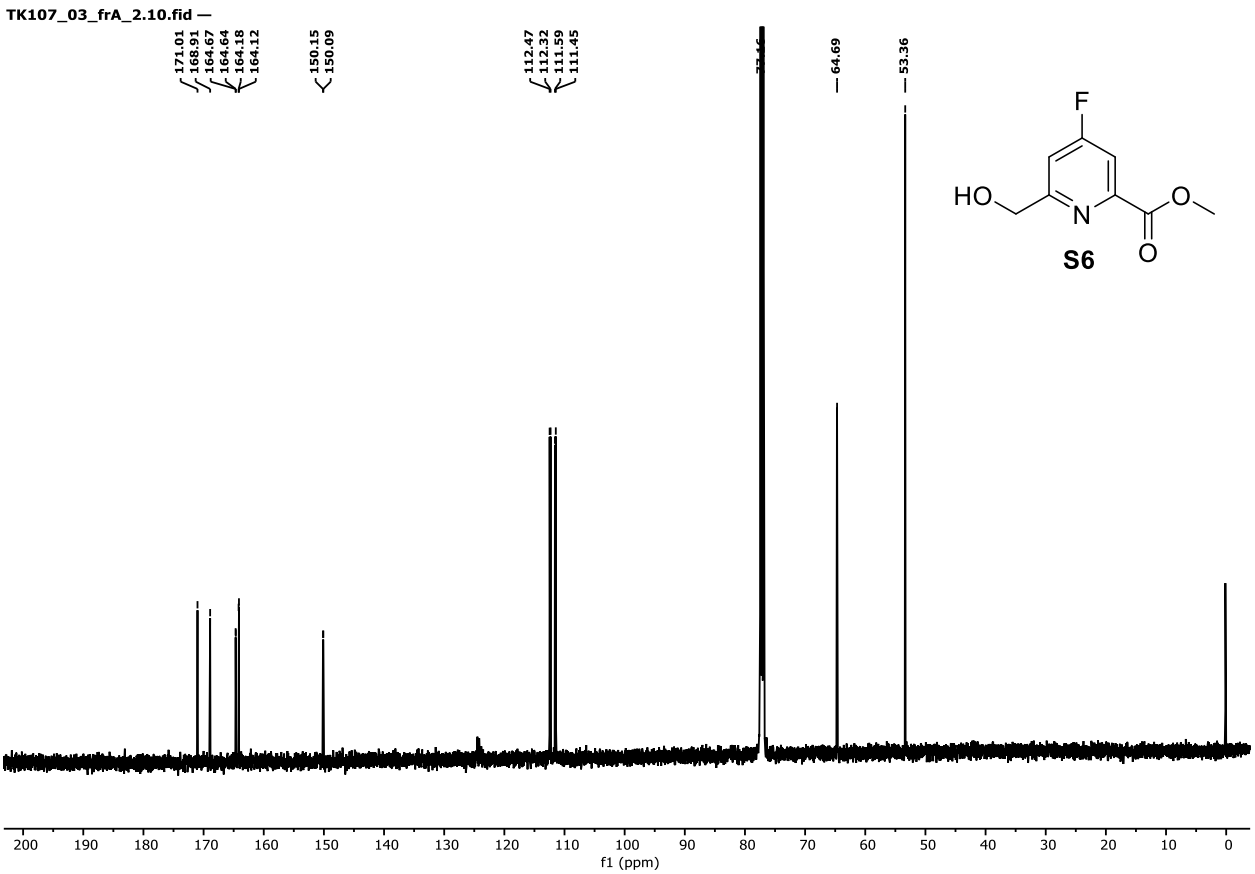


Figure S30: $^{13}C\{^{1}H\}$ NMR spectrum of **S6** in $CDCl_3$ (126 MHz, 298 K).

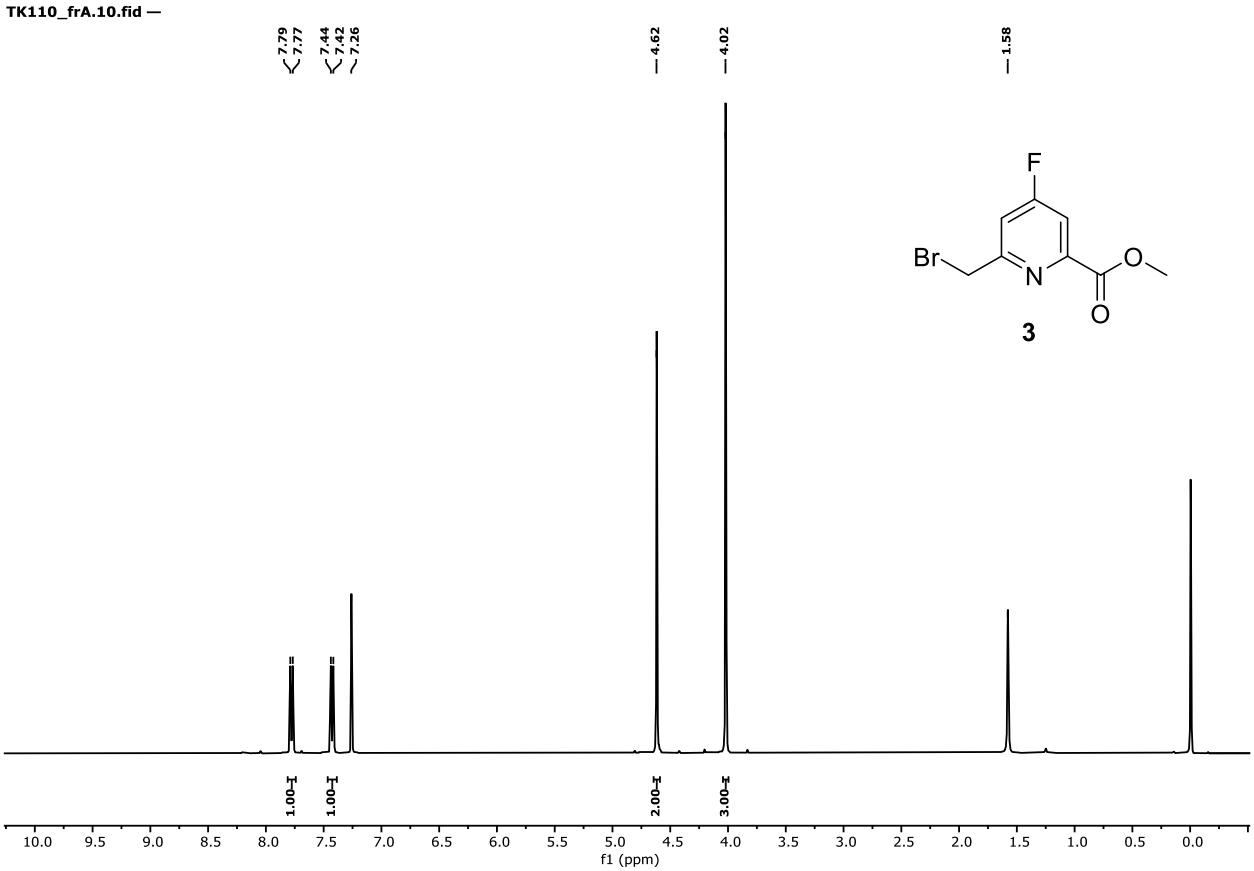


Figure S31: ^{1}H NMR spectrum of **3** in $CDCl_3$ (400 MHz, 298 K).

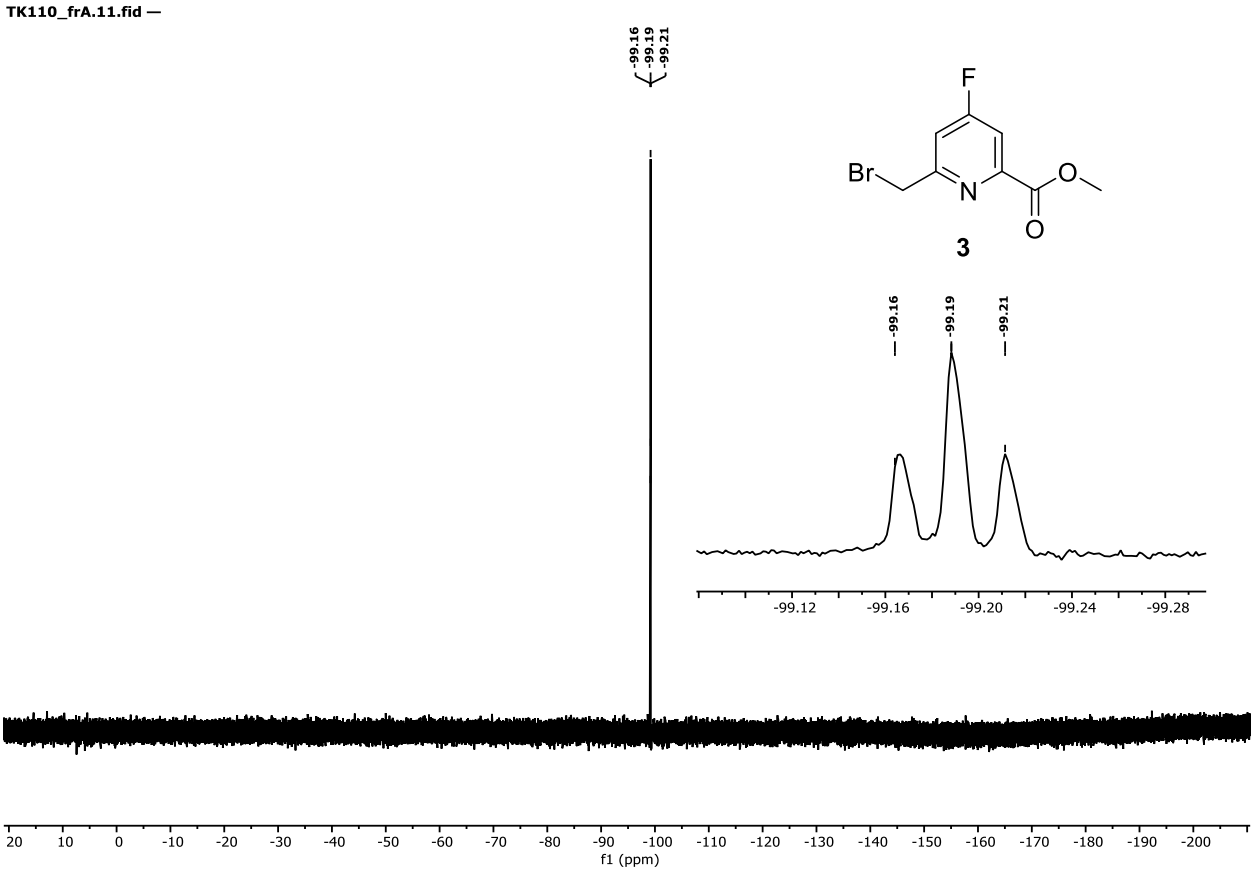


Figure S32: ^{19}F NMR spectrum of **3** in $CDCl_3$ (376 MHz, 298 K).

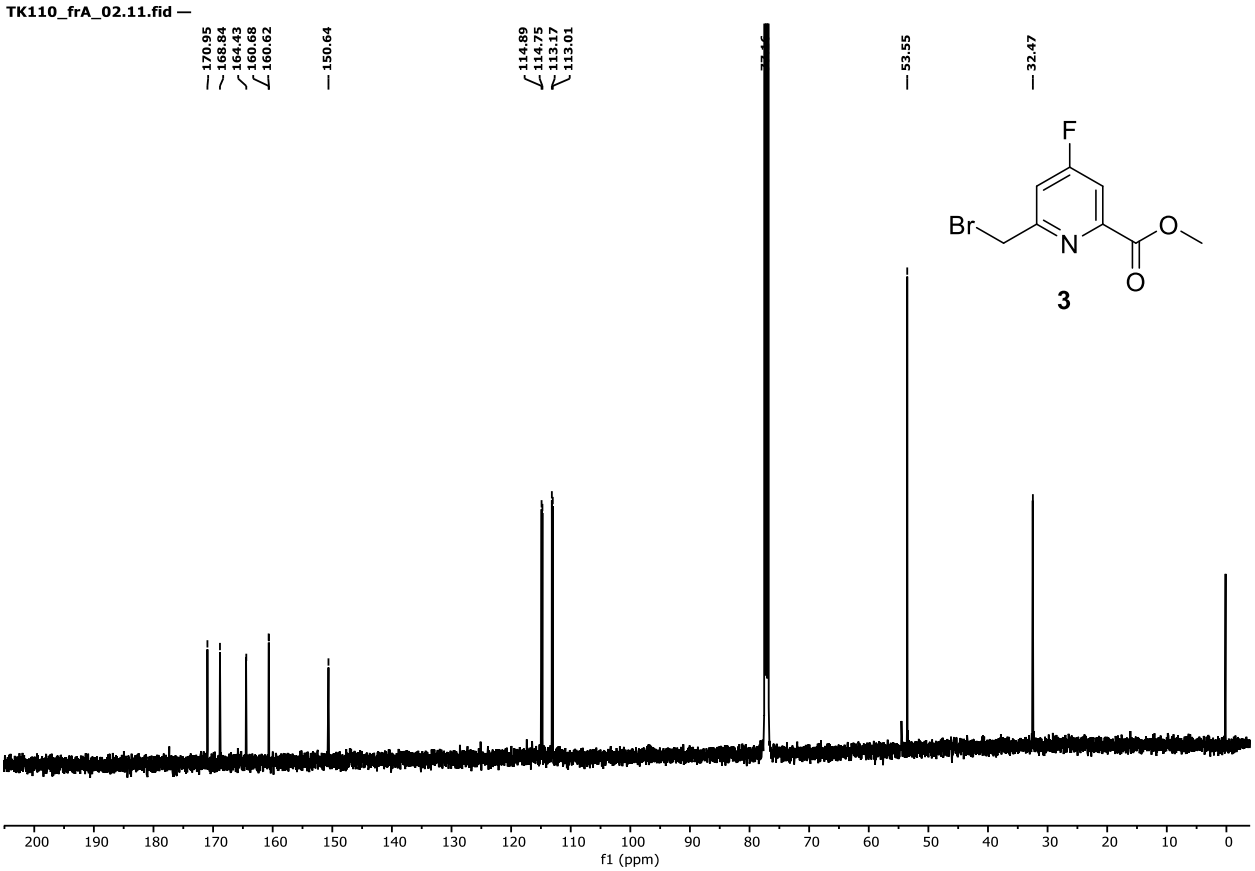


Figure S33: $^{13}C\{^{1}H\}$ NMR spectrum of **3** in $CDCl_3$ (126 MHz, 298 K).

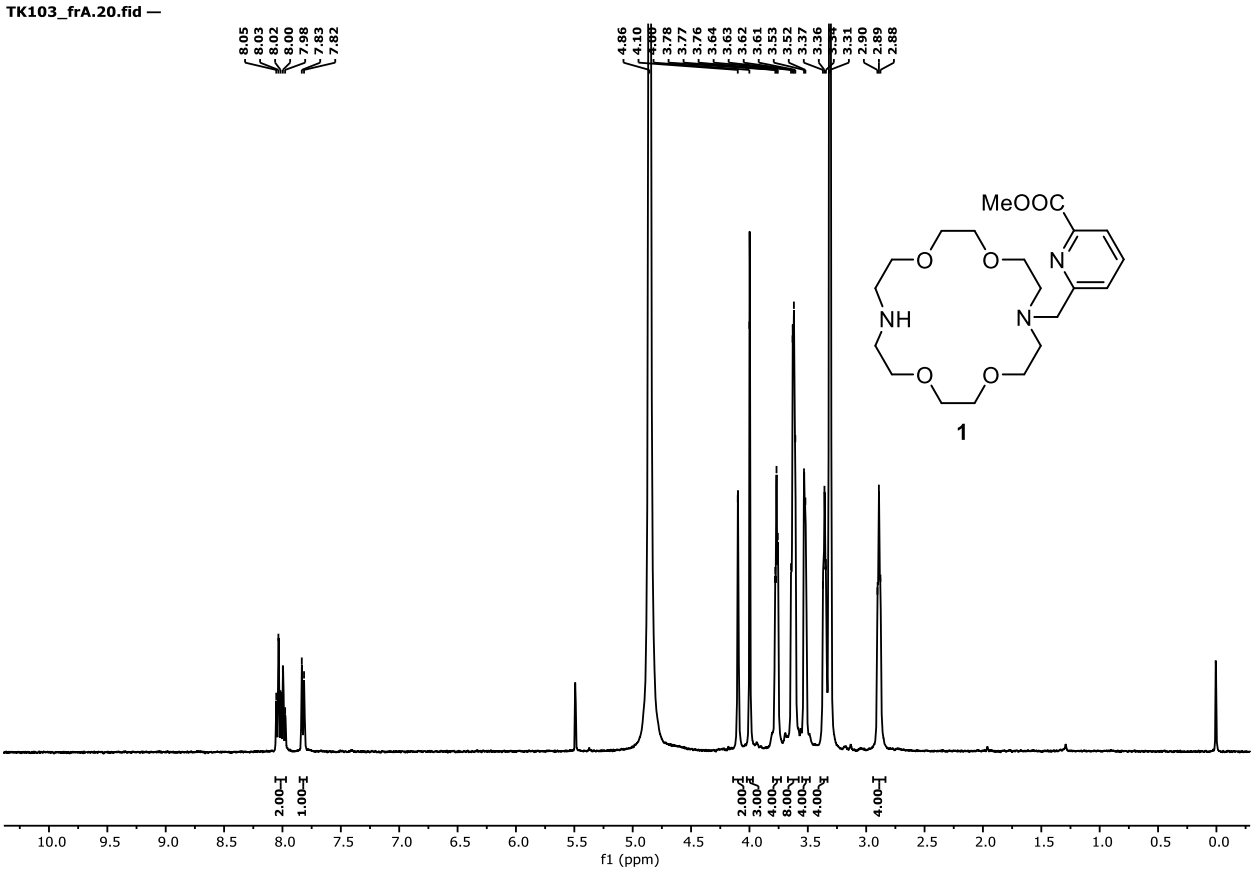


Figure S34: ^{1}H NMR spectrum of **1** in CD_3OD (400 MHz, 298 K).

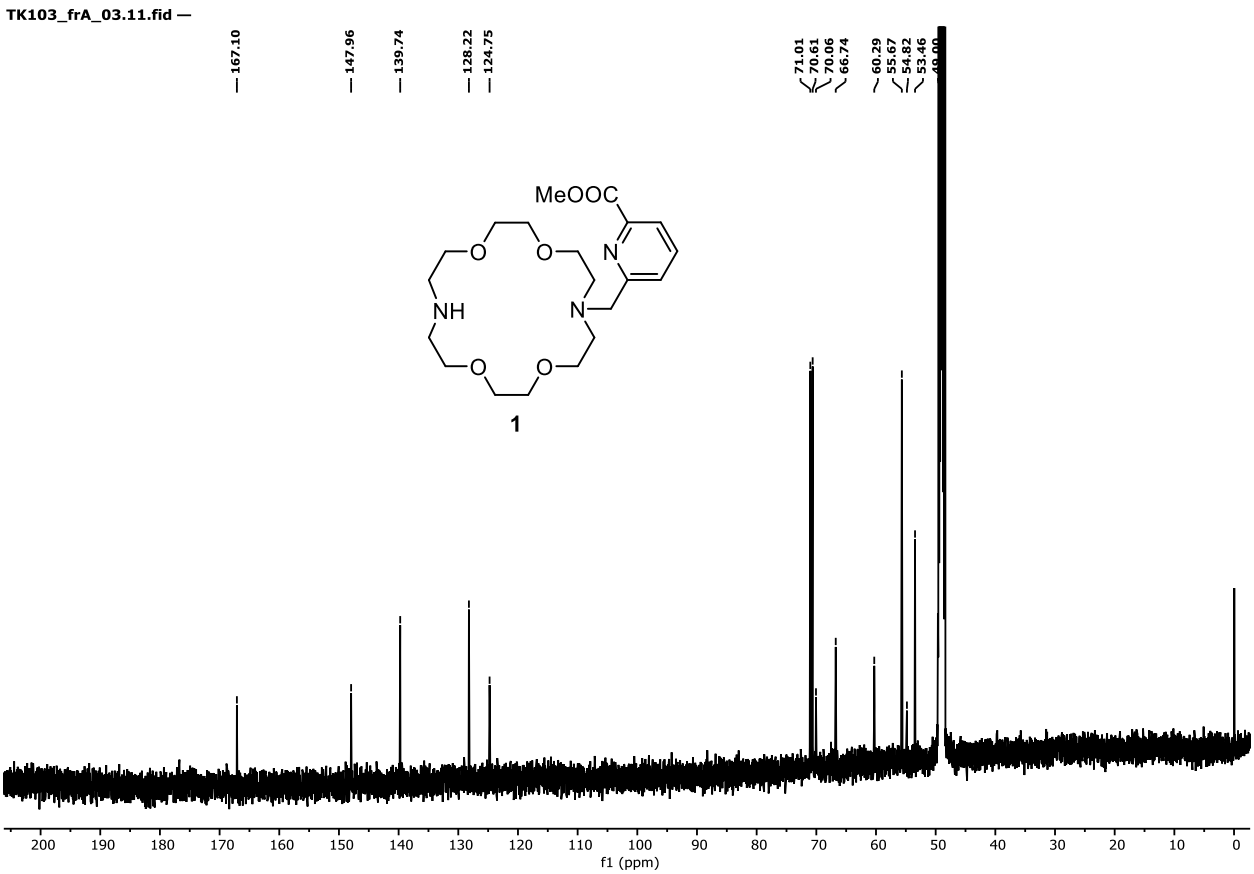


Figure S35: $^{13}C\{^{1}H\}$ NMR spectrum of **1** in CD_3OD (126 MHz, 298 K).

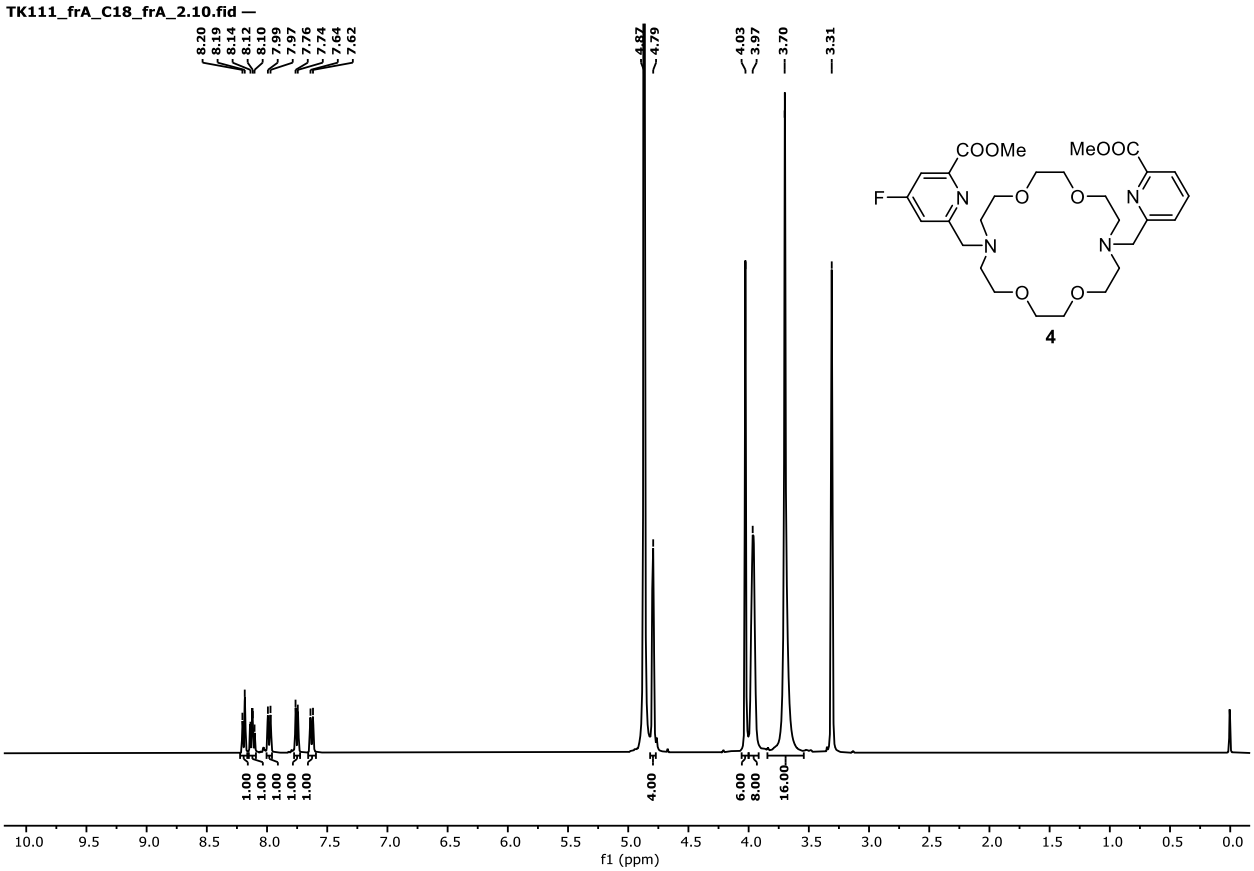


Figure S36: ^{1}H NMR spectrum of **4** in CD_3OD (400 MHz, 298 K).

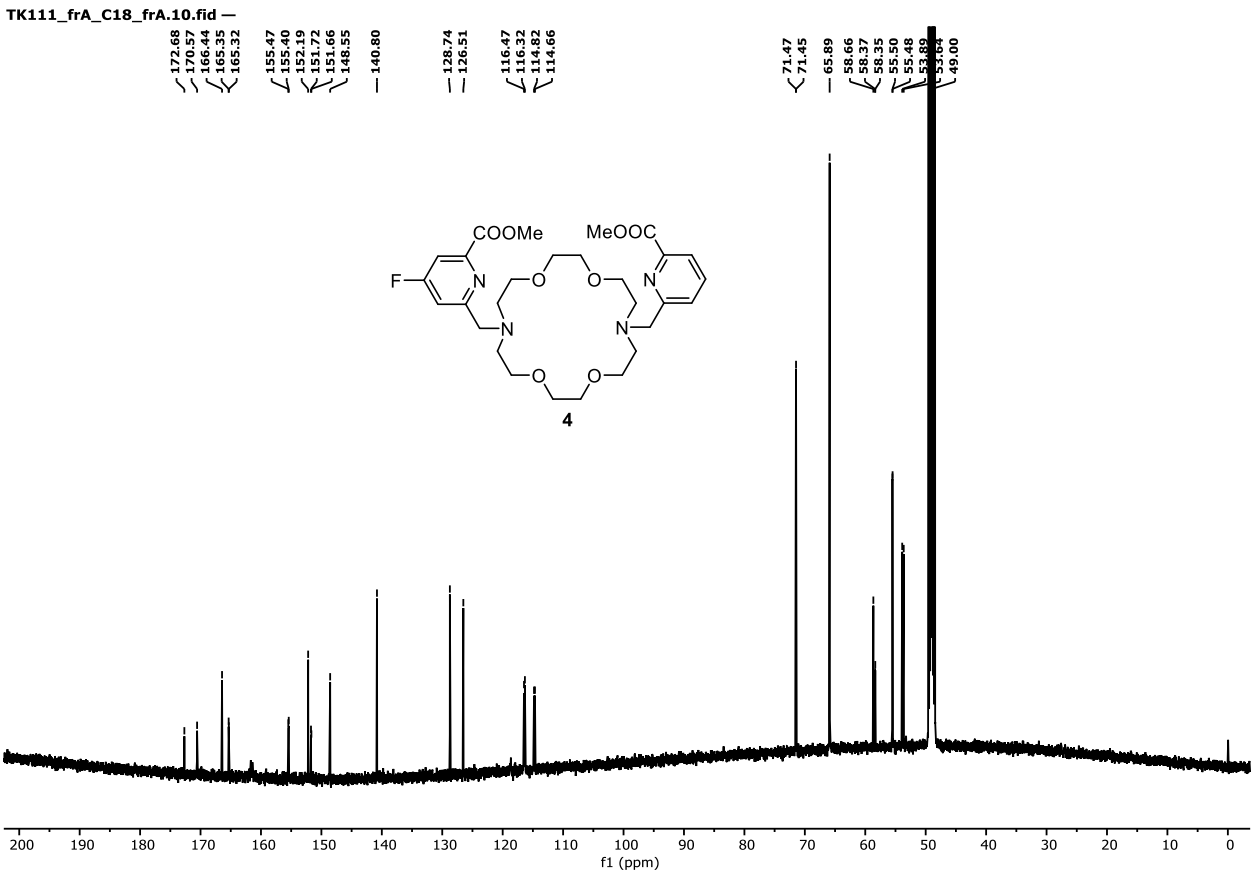


Figure S37: $^{13}C\{^{1}H\}$ NMR spectrum of **4** in CD_3OD (126 MHz, 298 K).

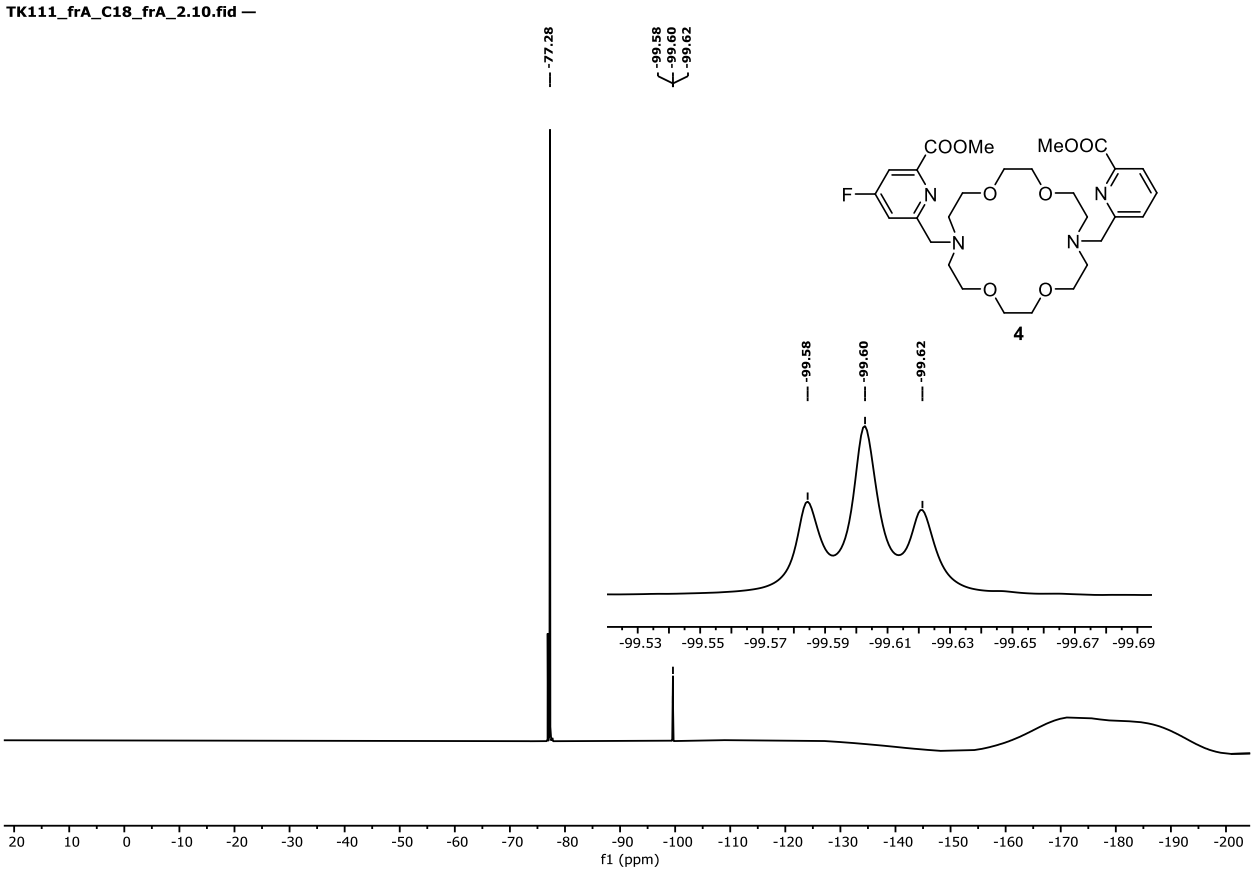


Figure S38: ^{19}F NMR spectrum of **4** in CD_3OD (470 MHz, 298 K).

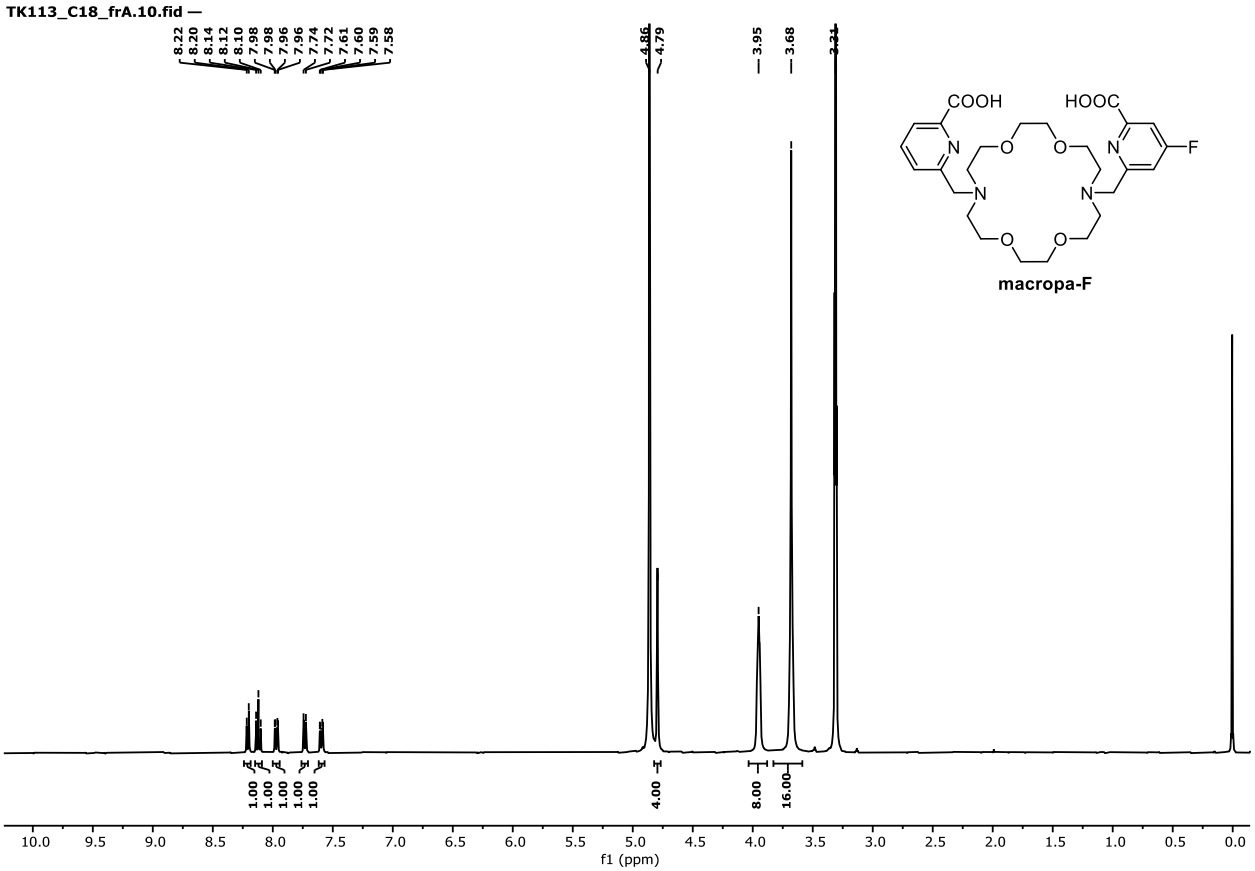


Figure S39: ^{1}H NMR spectrum of **macropa-F** in CD_3OD (400 MHz, 298 K).

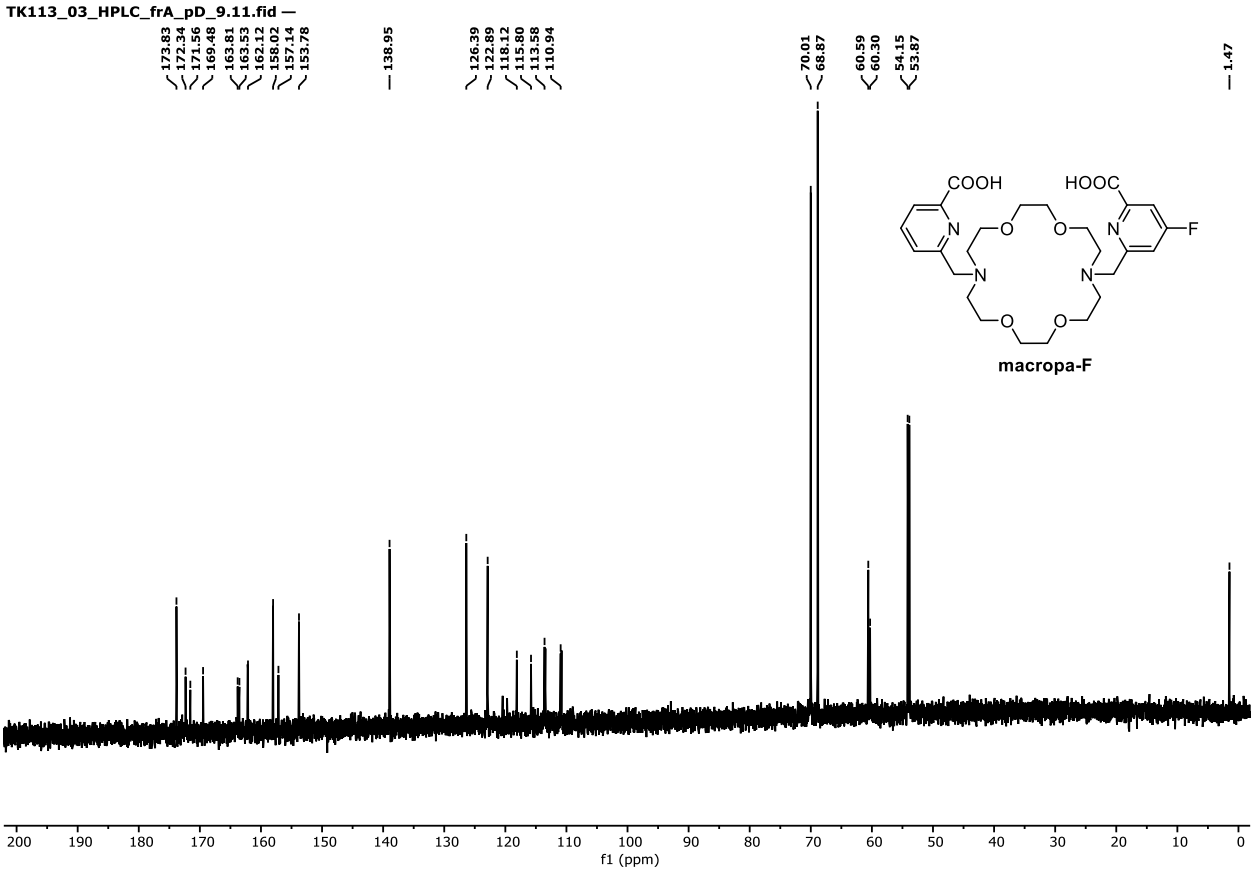


Figure S40: $^{13}C\{^{1}H\}$ NMR spectrum of **macropa-F** in D_2O (126 MHz, 298 K).

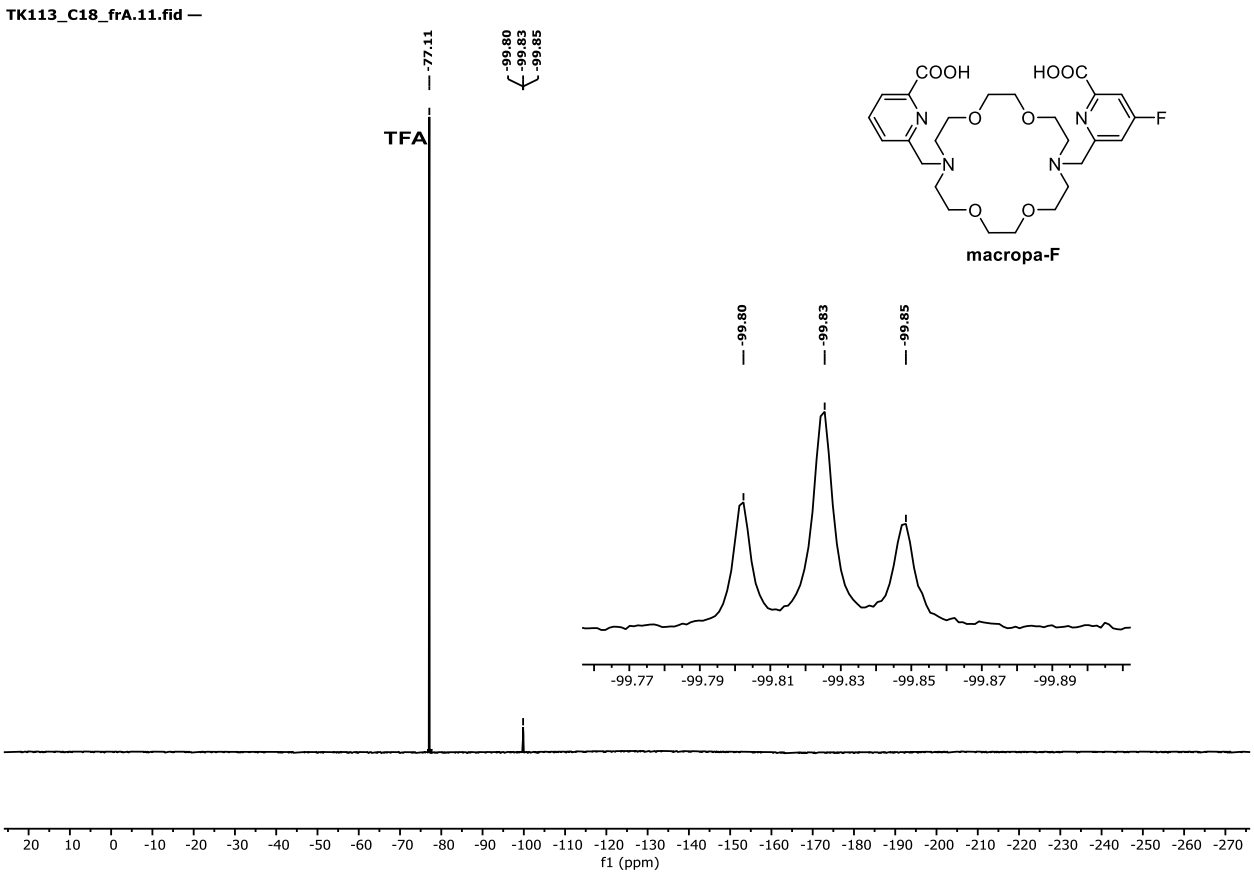


Figure S41: ^{19}F NMR spectrum of **macropa-F** in CD_3OD (376 MHz, 298 K).

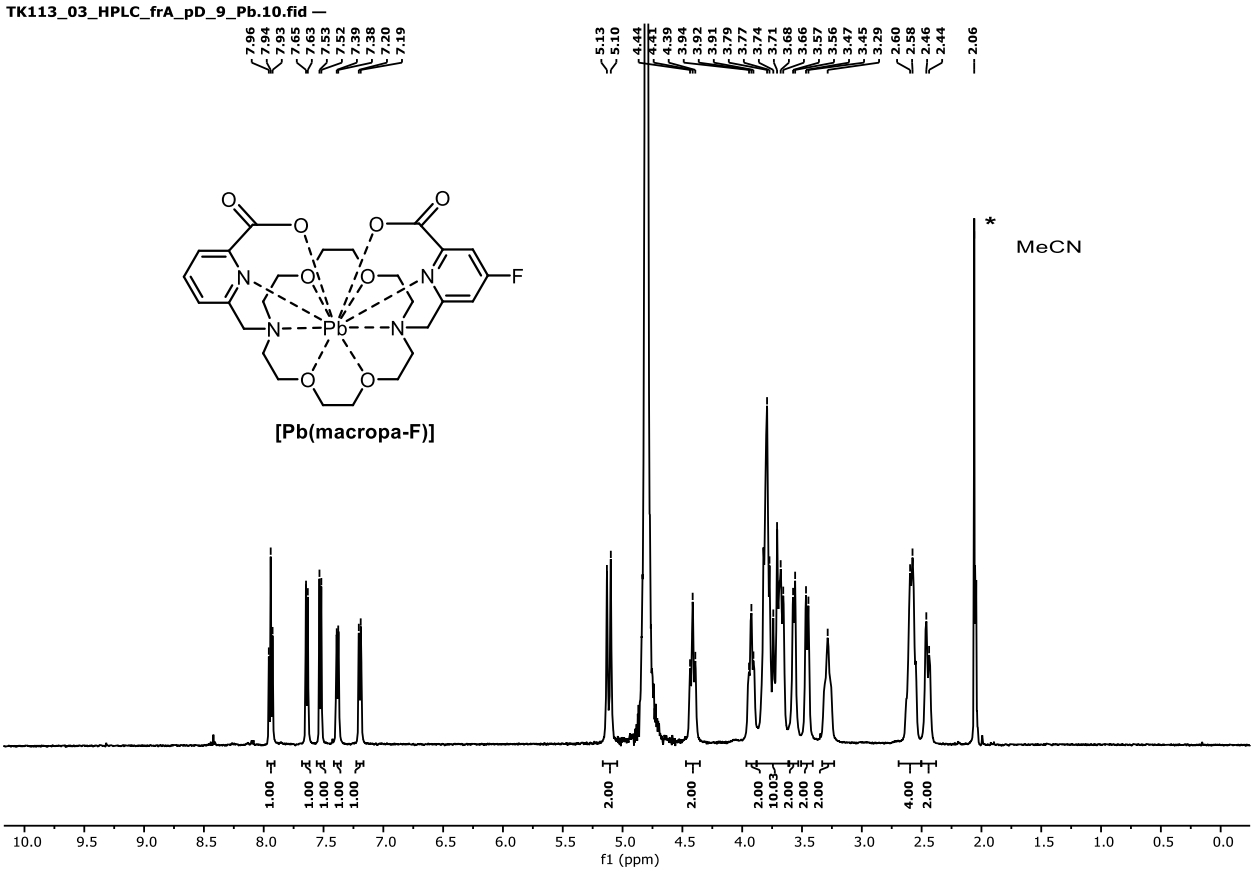


Figure S42: ^{1}H NMR spectrum of **[Pb(macropa-F)]** in D_2O, spiked with MeCN* (500 MHz, 298 K).

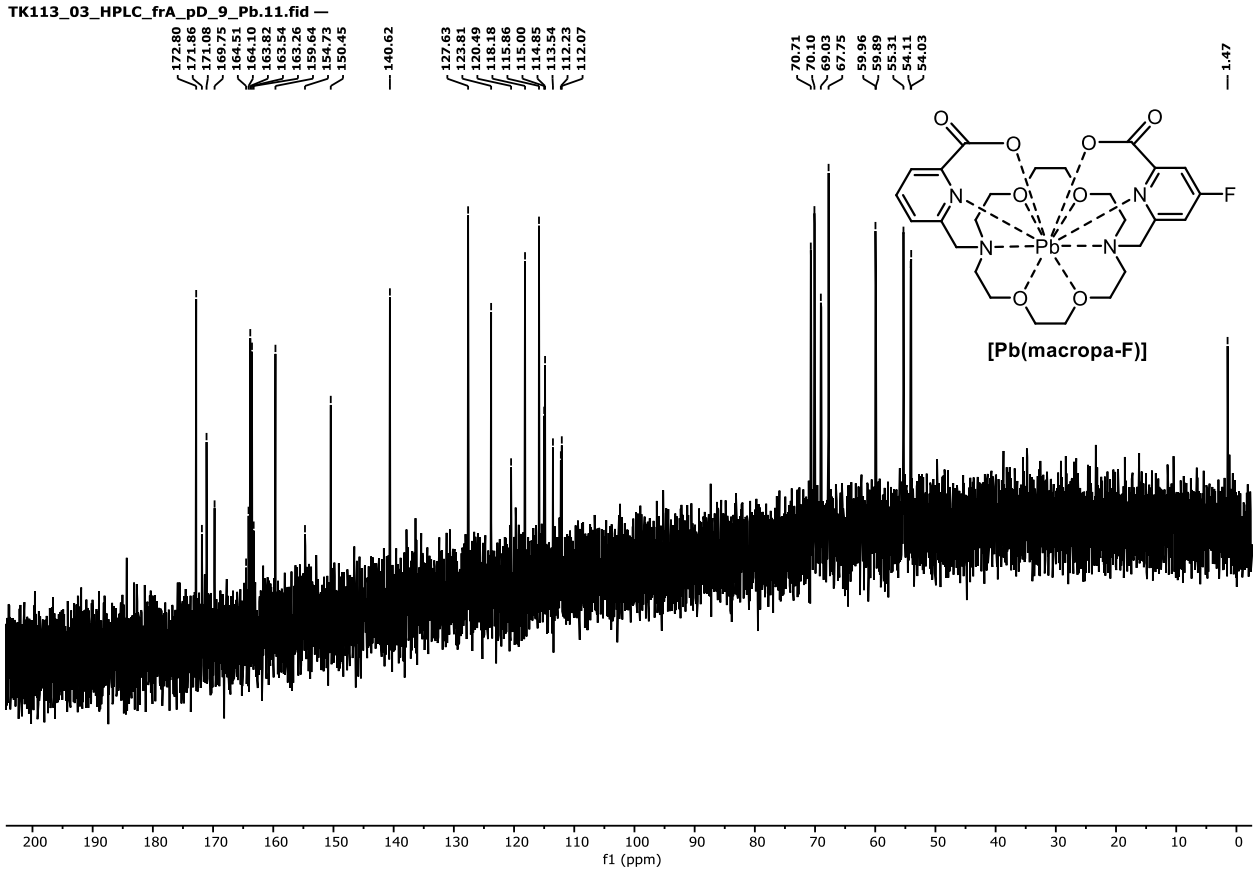


Figure S43: $^{13}C\{^{1}H\}$ NMR spectrum of **[Pb(macropa-F)]** in D_2O, spiked with MeCN (126 MHz, 298 K).

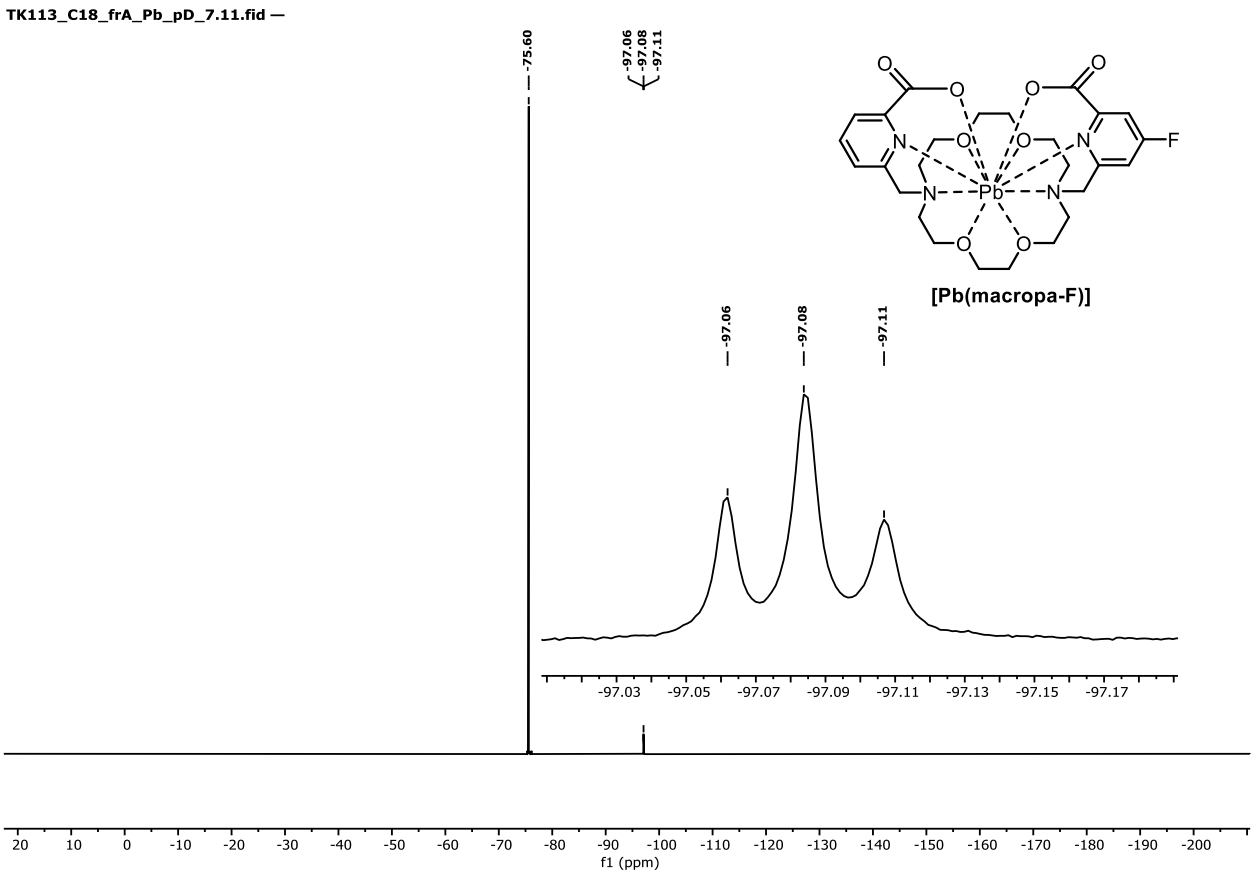


Figure S44: ^{19}F NMR spectrum of **[Pb(macropa-F)]** in D_2O, spiked with MeCN* (376 MHz, 298 K).

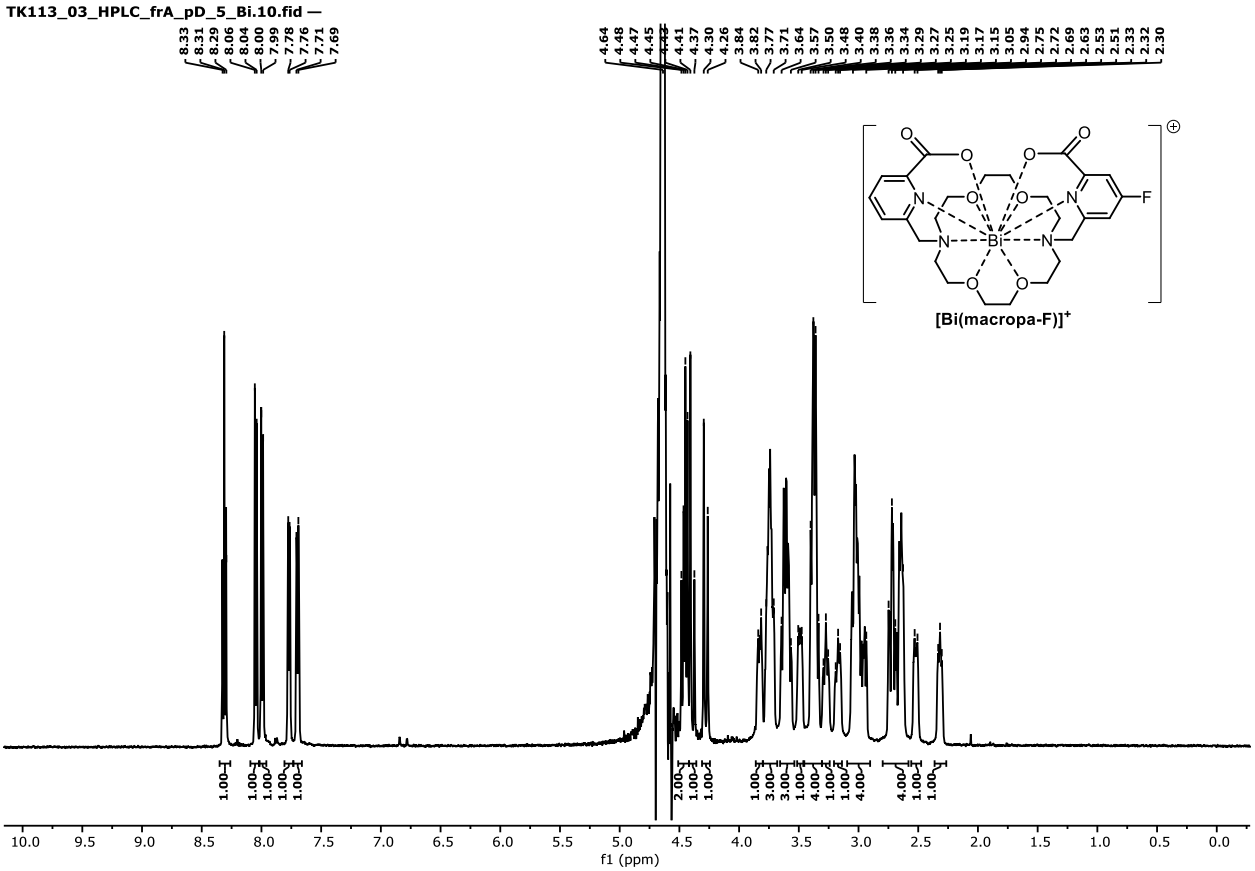


Figure S45: ^{1}H NMR spectrum of **[Bi(macropa-F)]$^{+}$** in D_2O, spiked with MeCN* (500 MHz, 298 K).

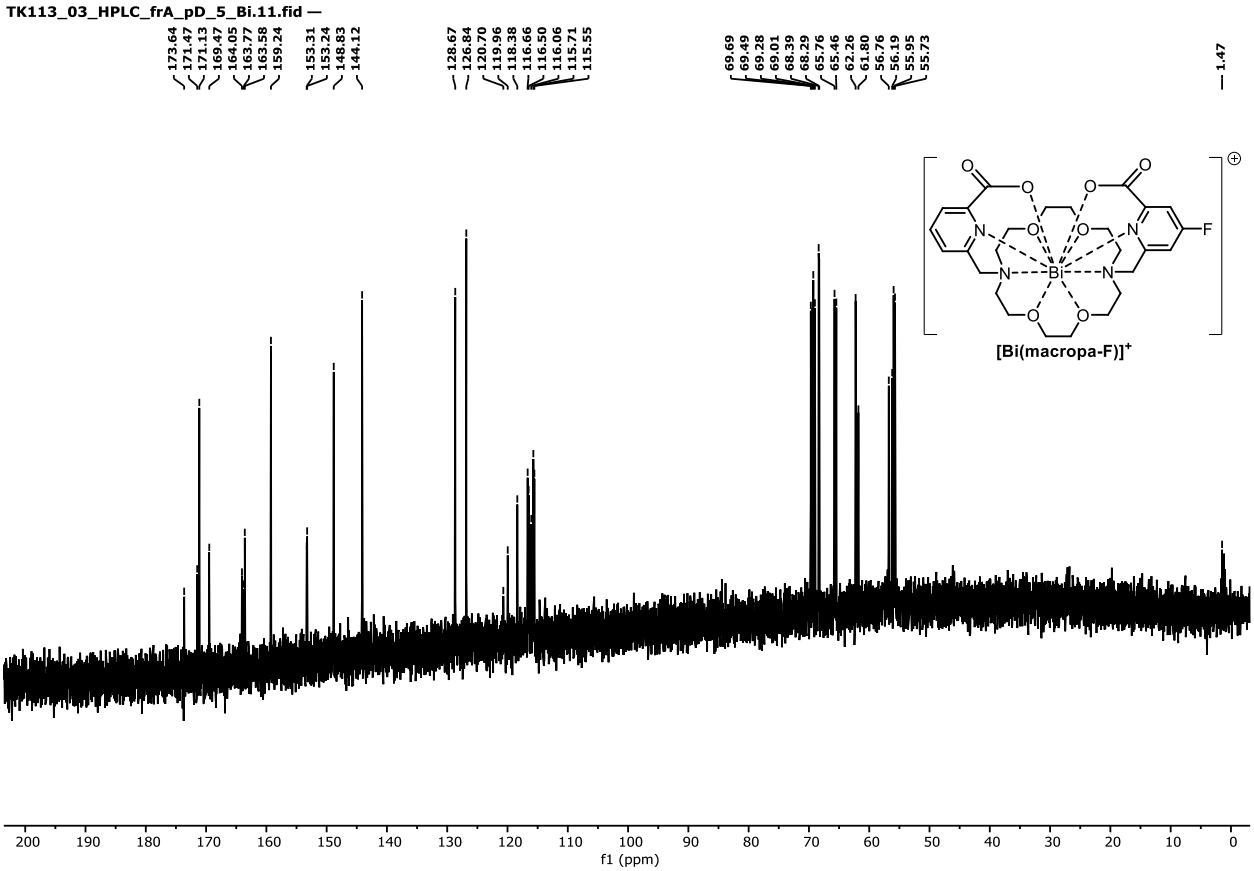


Figure S46: $^{13}C\{^{1}H\}$ NMR spectrum of **[Bi(macropa-F)]$^{+}$** in D_2O, spiked with MeCN (126 MHz, 298 K).

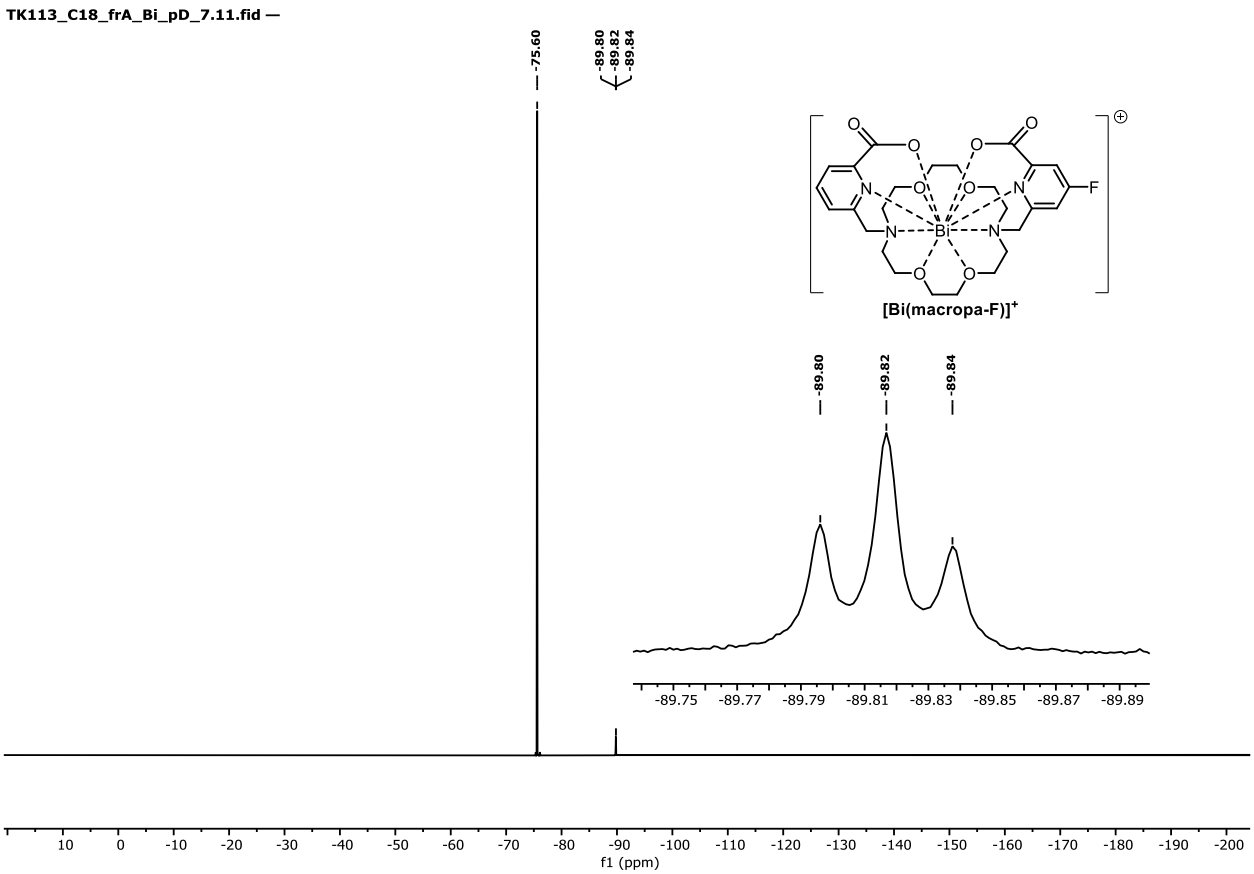


Figure S47: ^{19}F NMR spectrum of **[Bi(macropa-F)]$^+$** in D_2O, spiked with MeCN* (376 MHz, 298 K).

9.2 HR-DART-MS and HR-ESI-MS

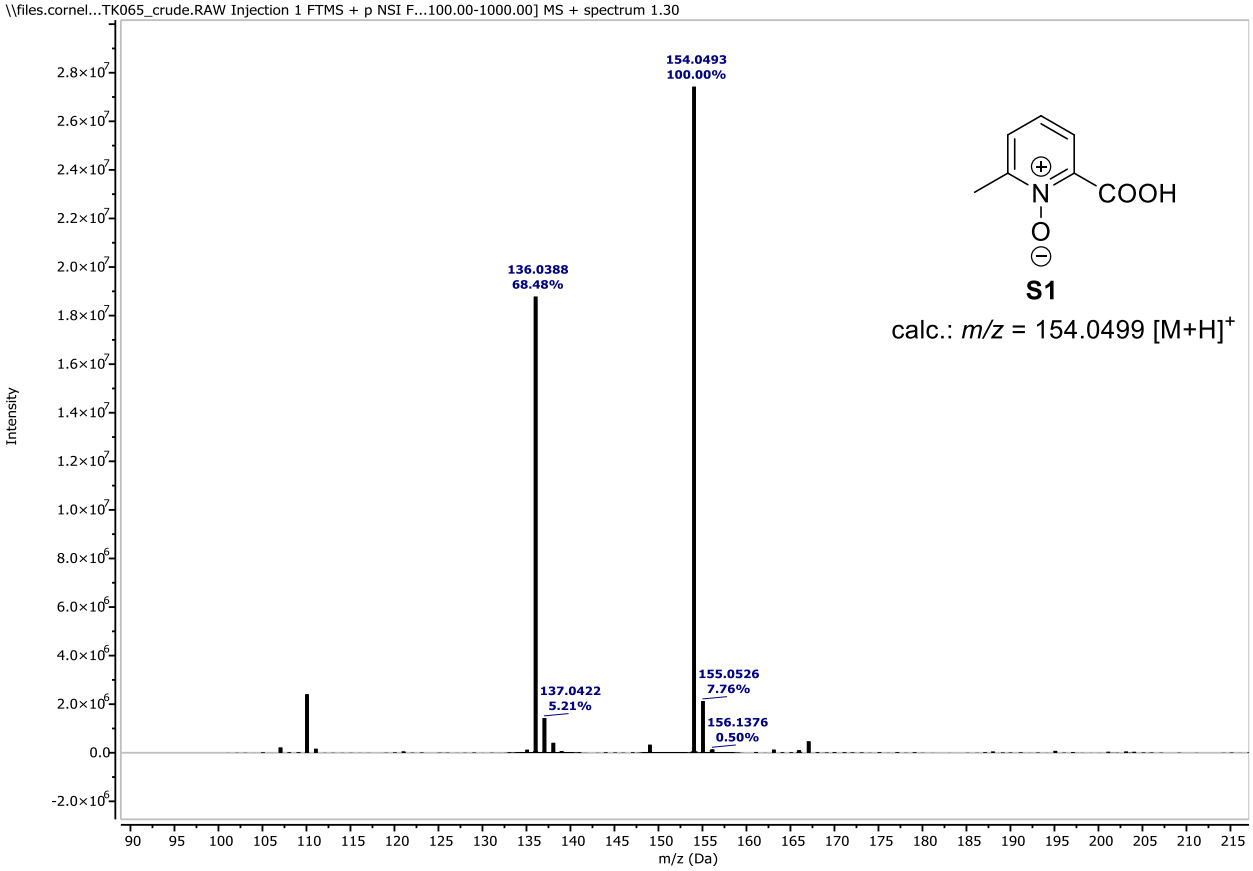


Figure S48: Positive HR-DART-MS of **S1**.

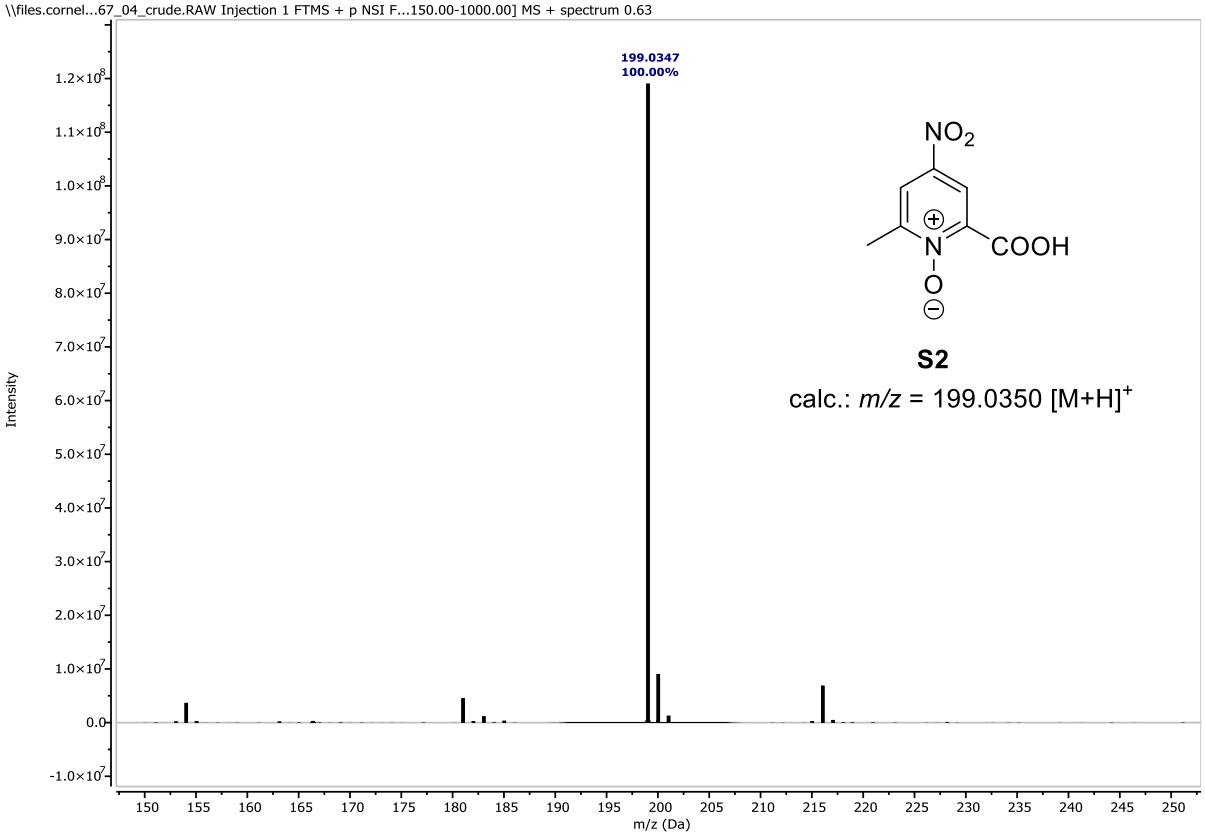


Figure S49: Positive HR-DART-MS of **S2**.

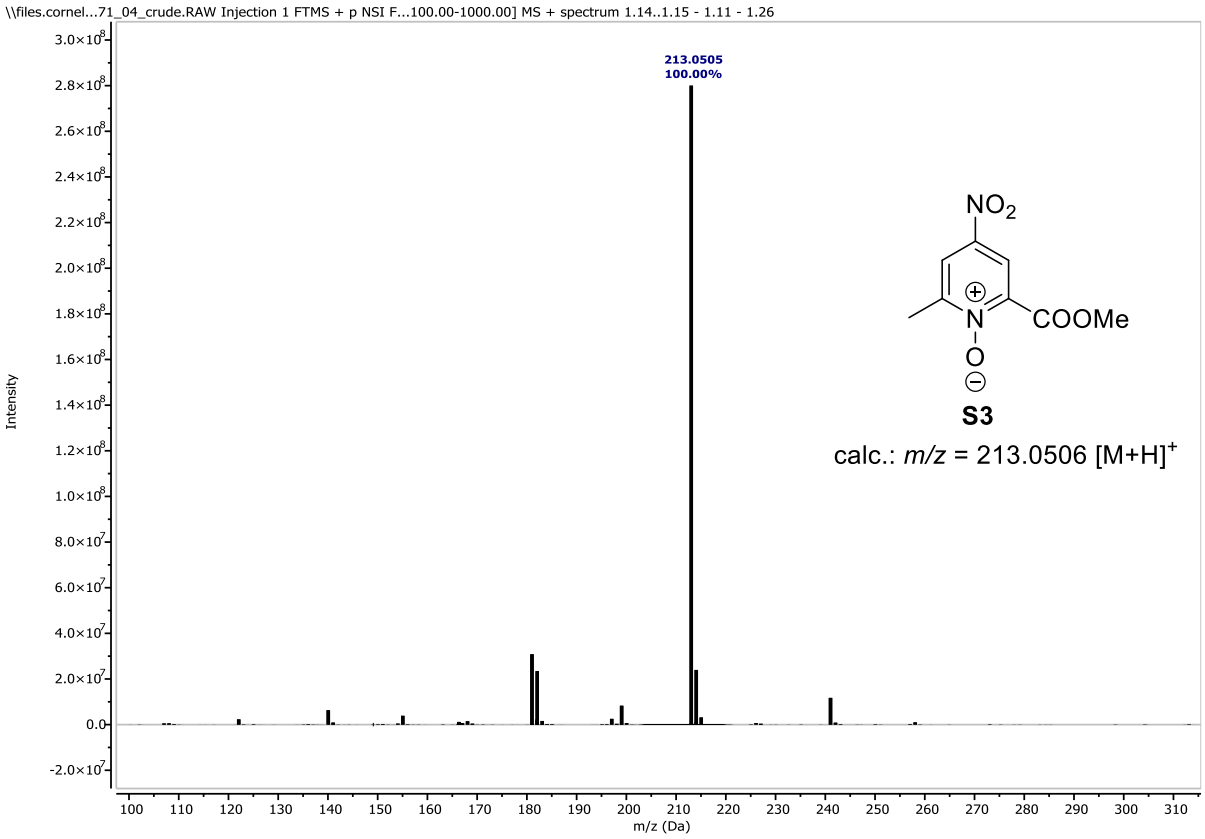


Figure S50: Positive HR-DART-MS of **S3**.

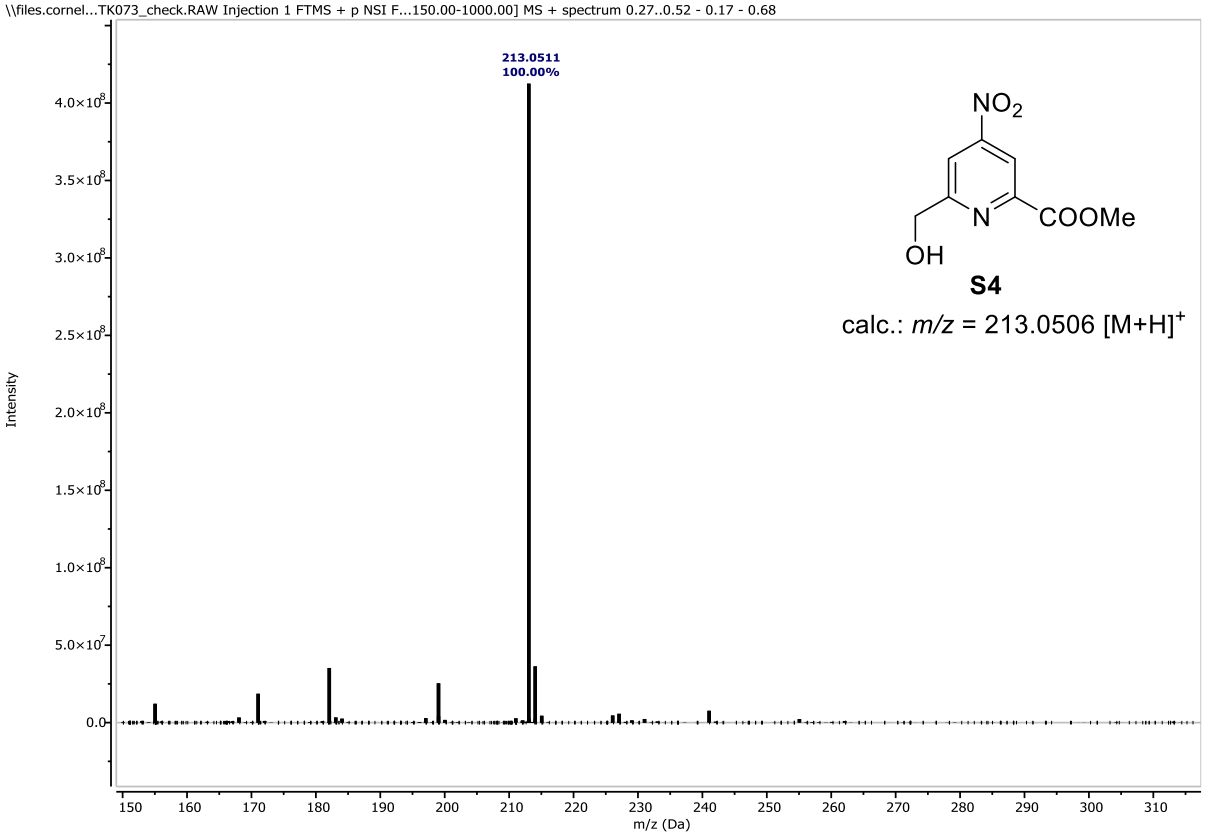


Figure S51: Positive HR-DART-MS of **S4**.

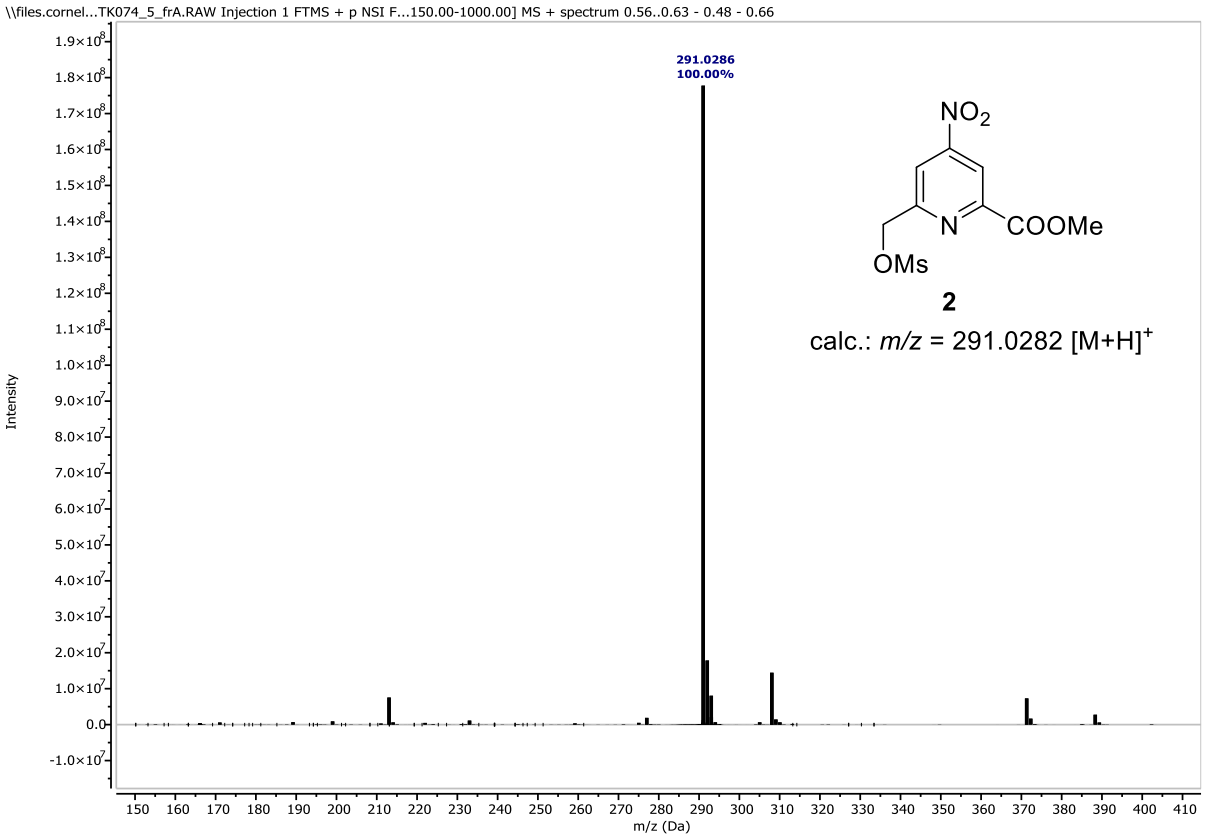


Figure S52: Positive HR-DART-MS of **2**.

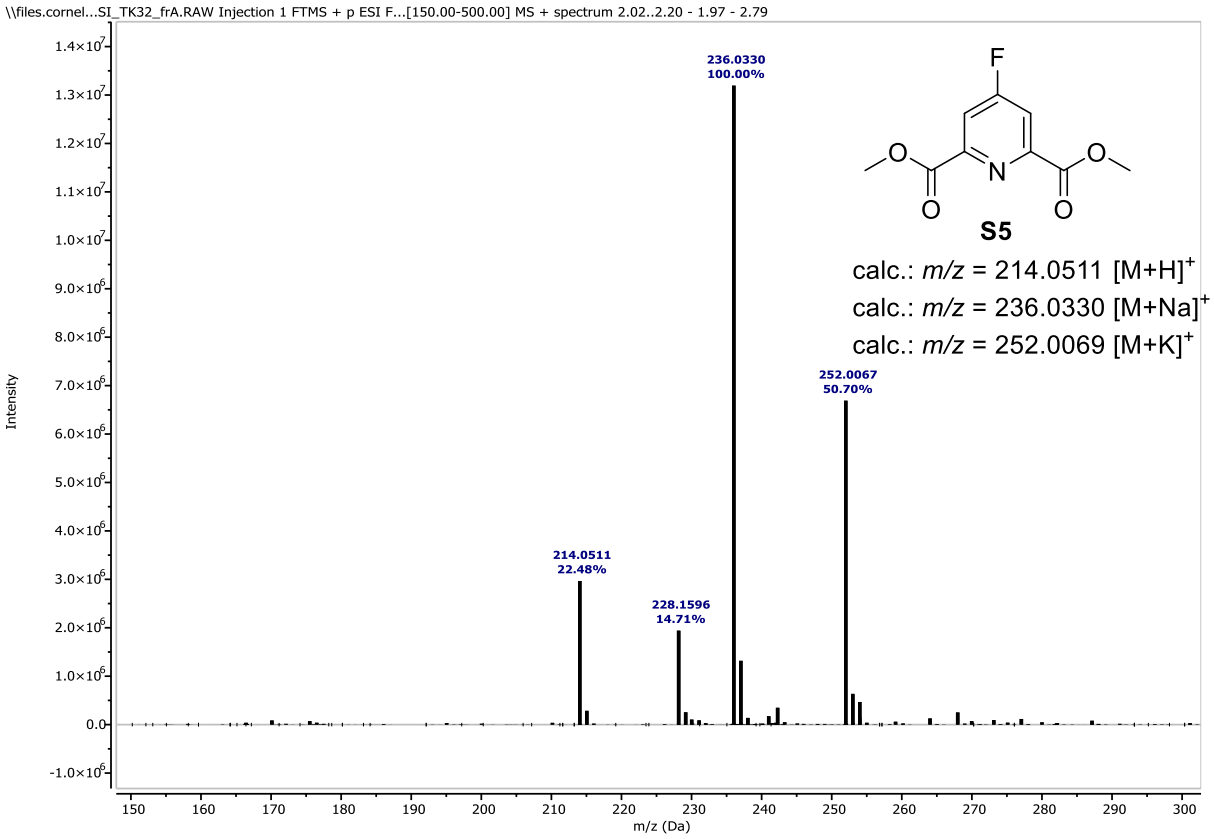


Figure S53: Positive ESI-MS of **S5** in acetonitrile.

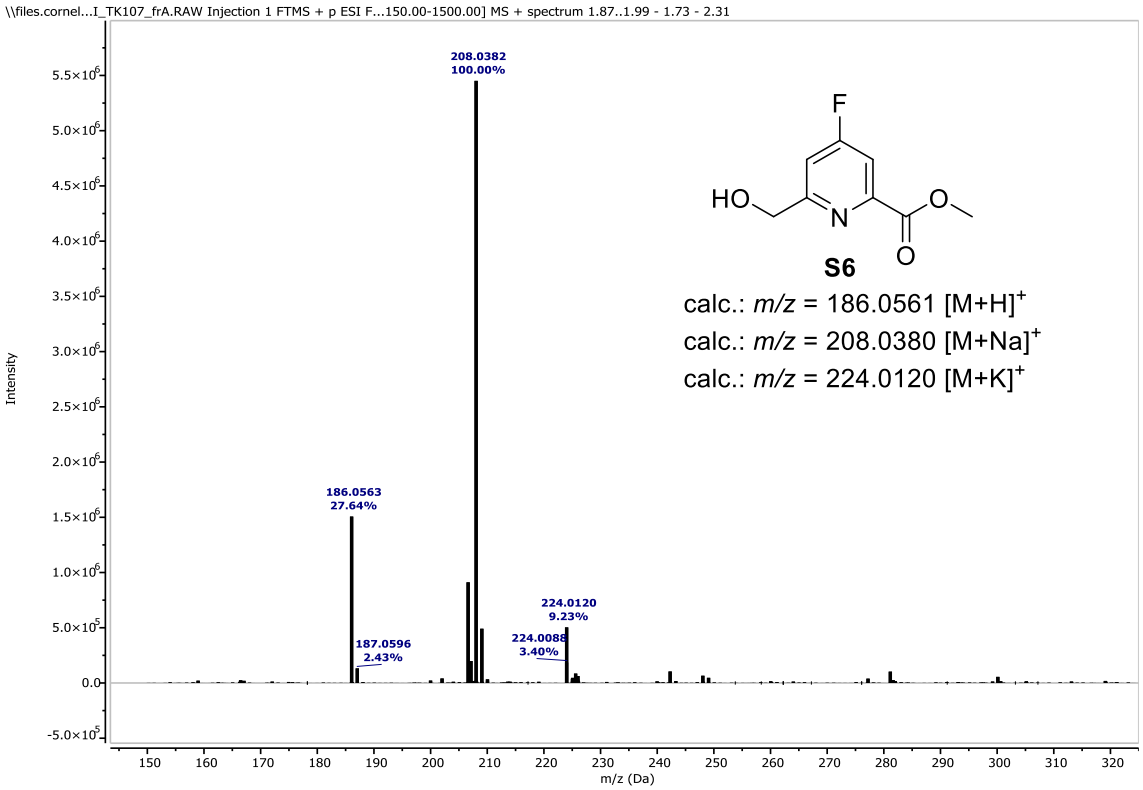


Figure S54: Positive ESI-MS of **S6** in acetonitrile.

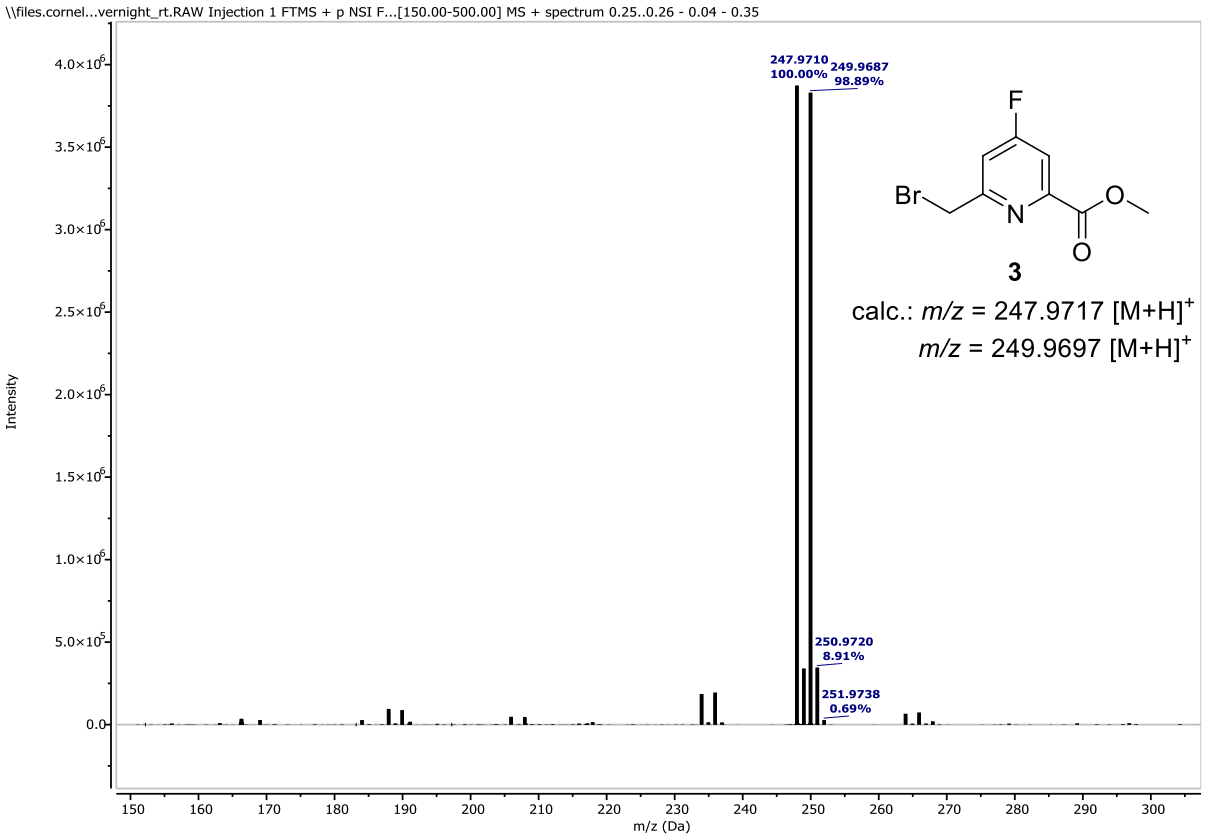


Figure S55: Positive HR-DART-MS of **3**.

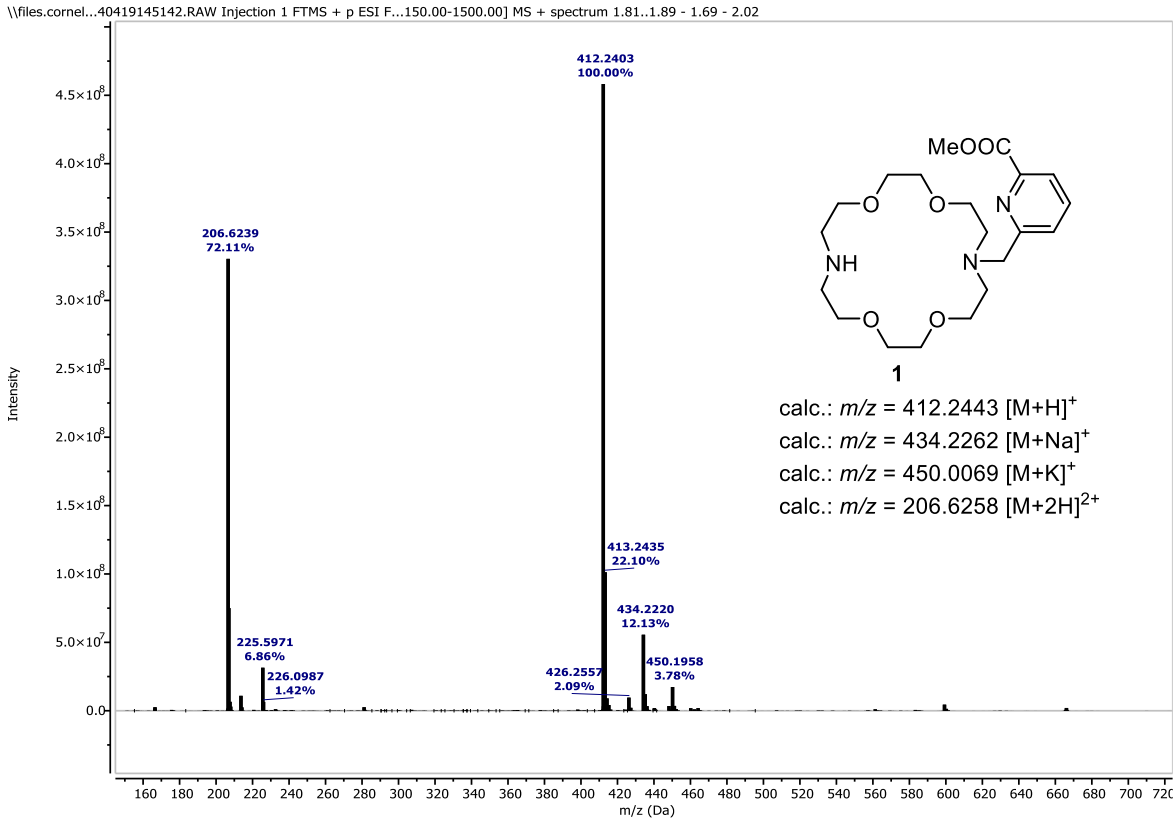


Figure S56: Positive ESI-MS of **1** in methanol.

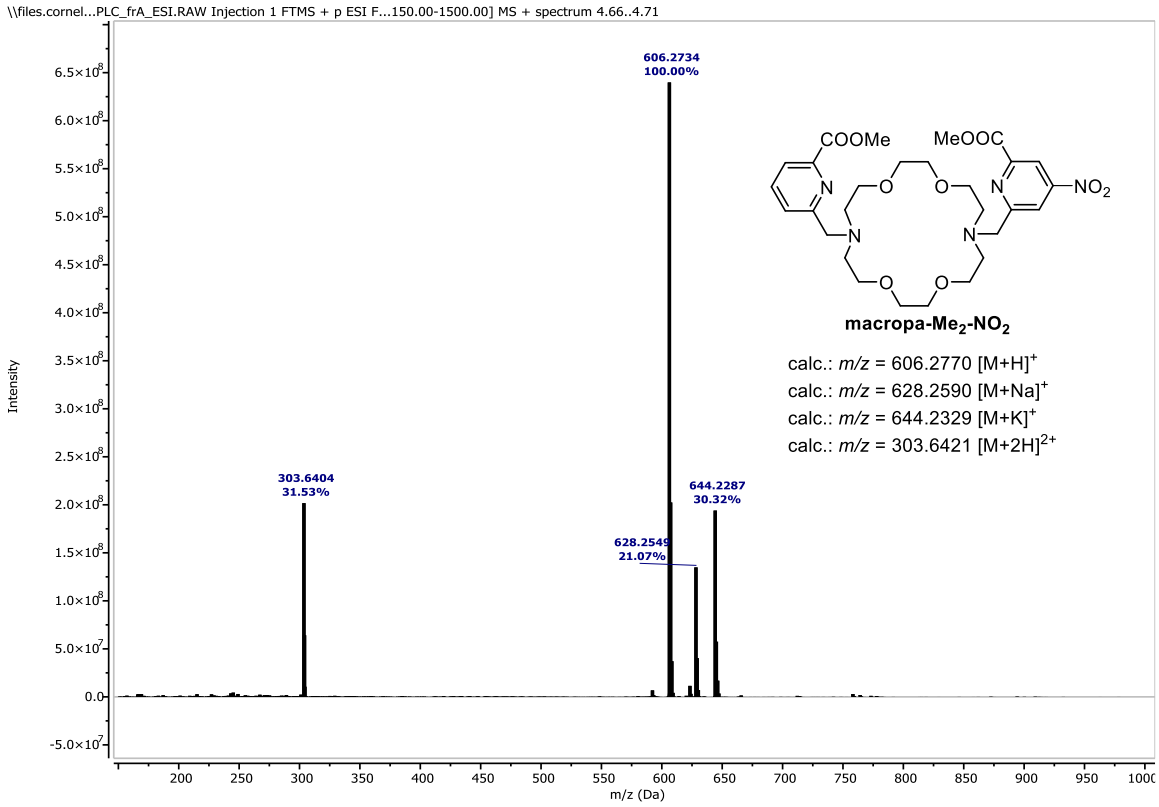


Figure S57: Positive ESI-MS of **macropa-Me$_2$-NO$_2$** in methanol.

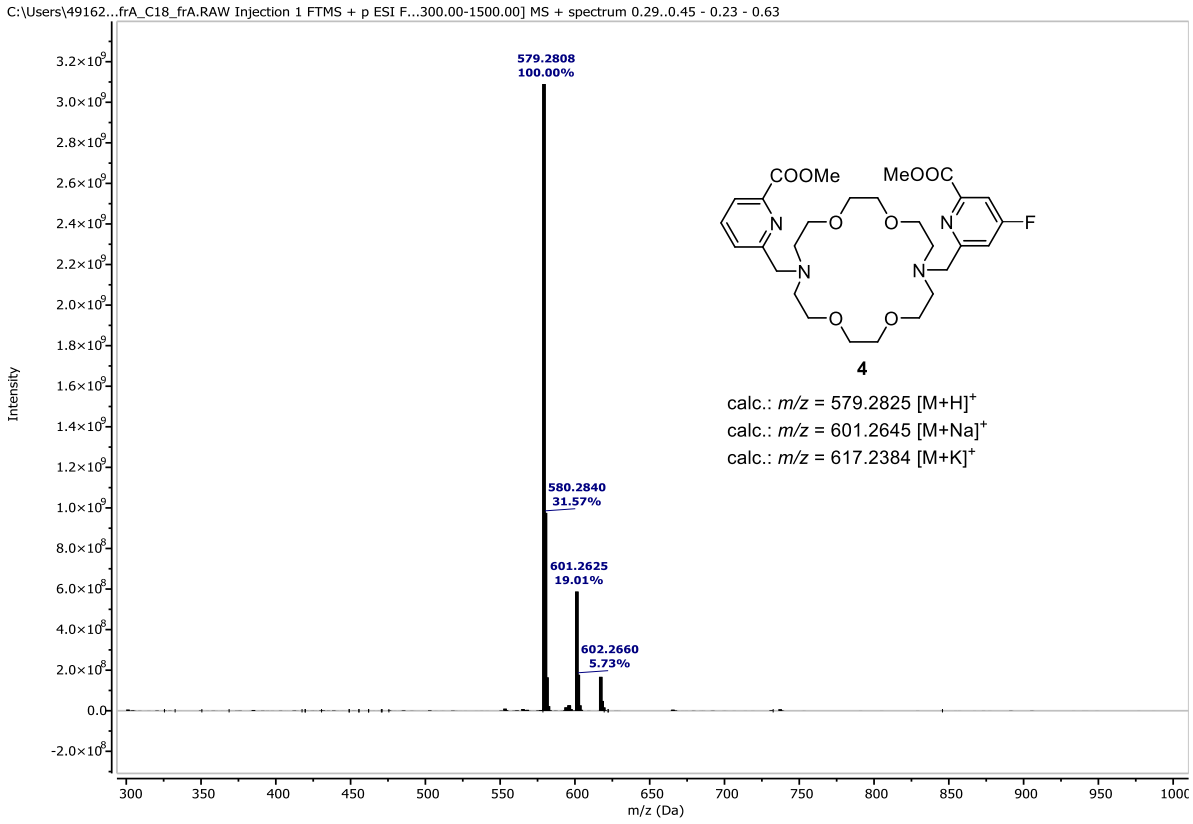


Figure S58: Positive ESI-MS of **4** in methanol.

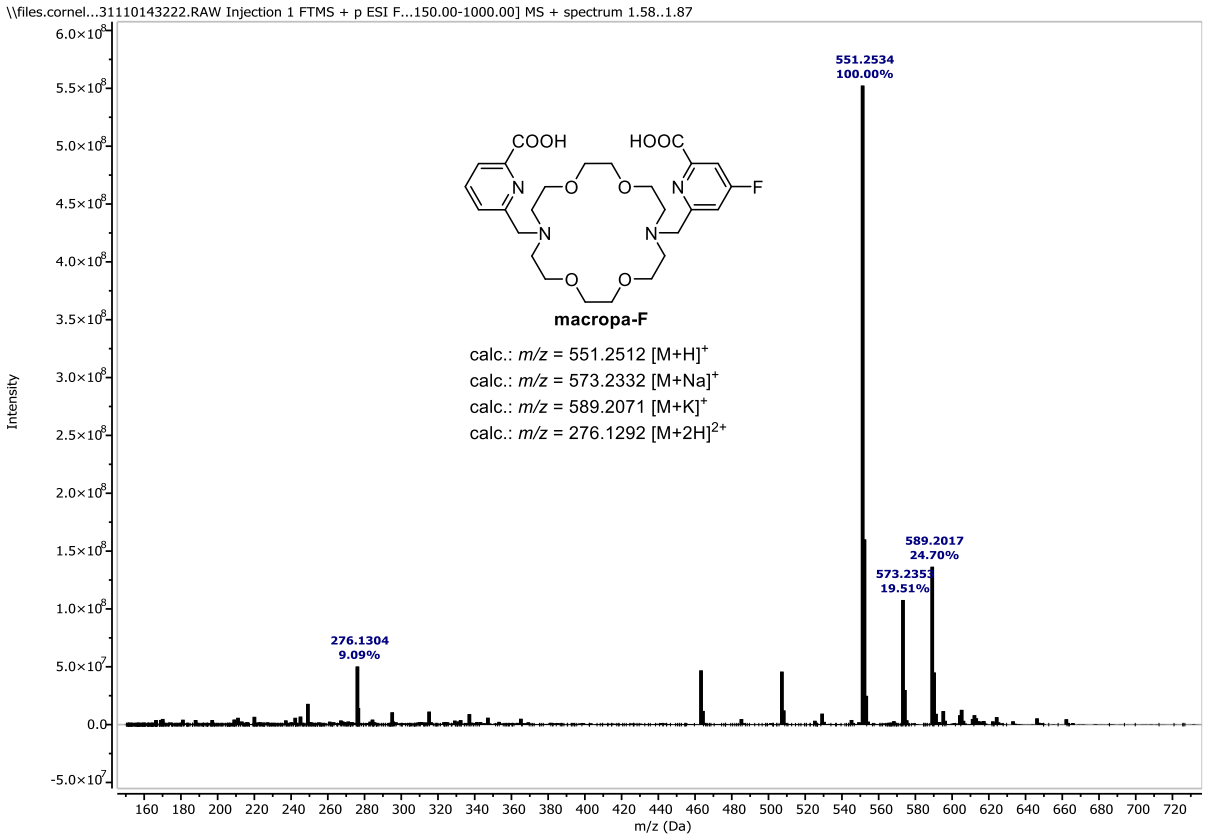


Figure S59: Positive ESI-MS of **macropa-F** in methanol.

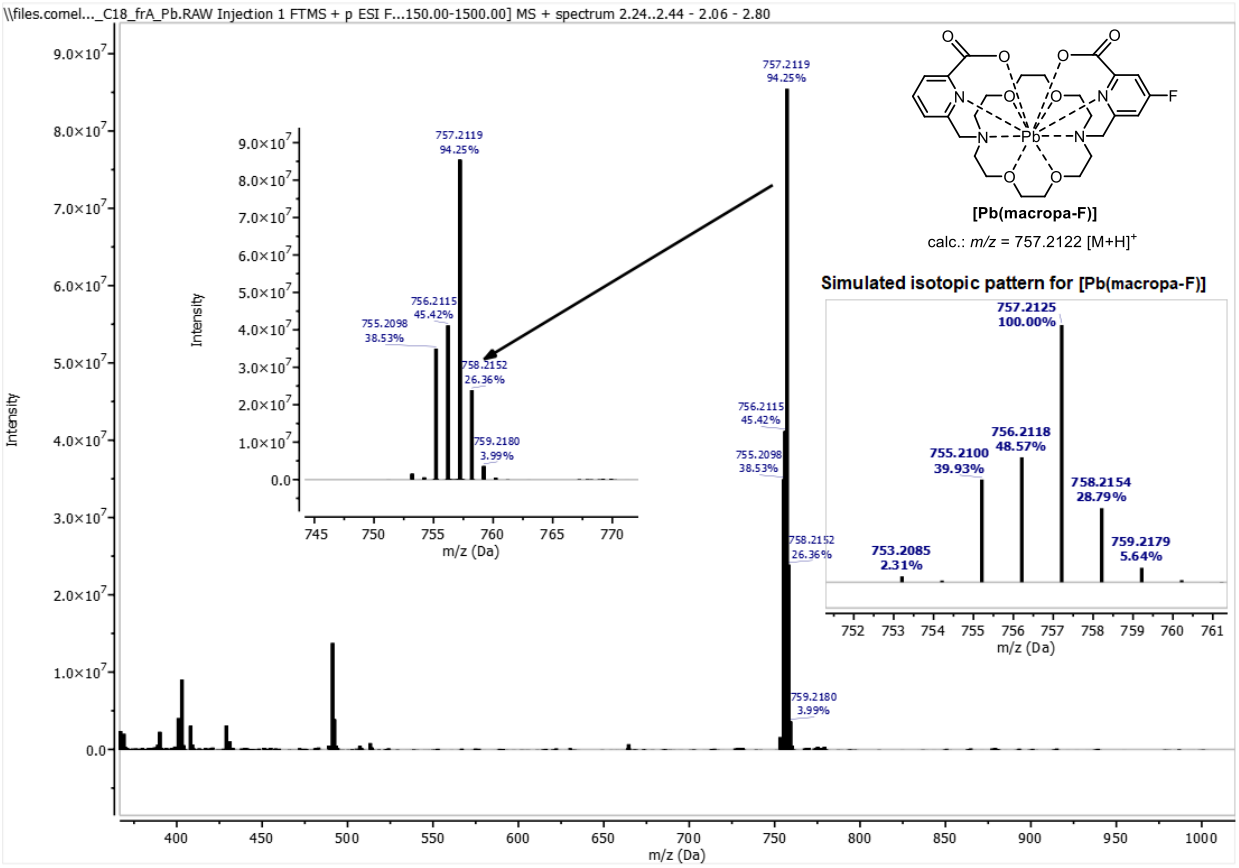


Figure S60: Positive ESI-MS of **[Pb(macropa-F)]** in methanol.

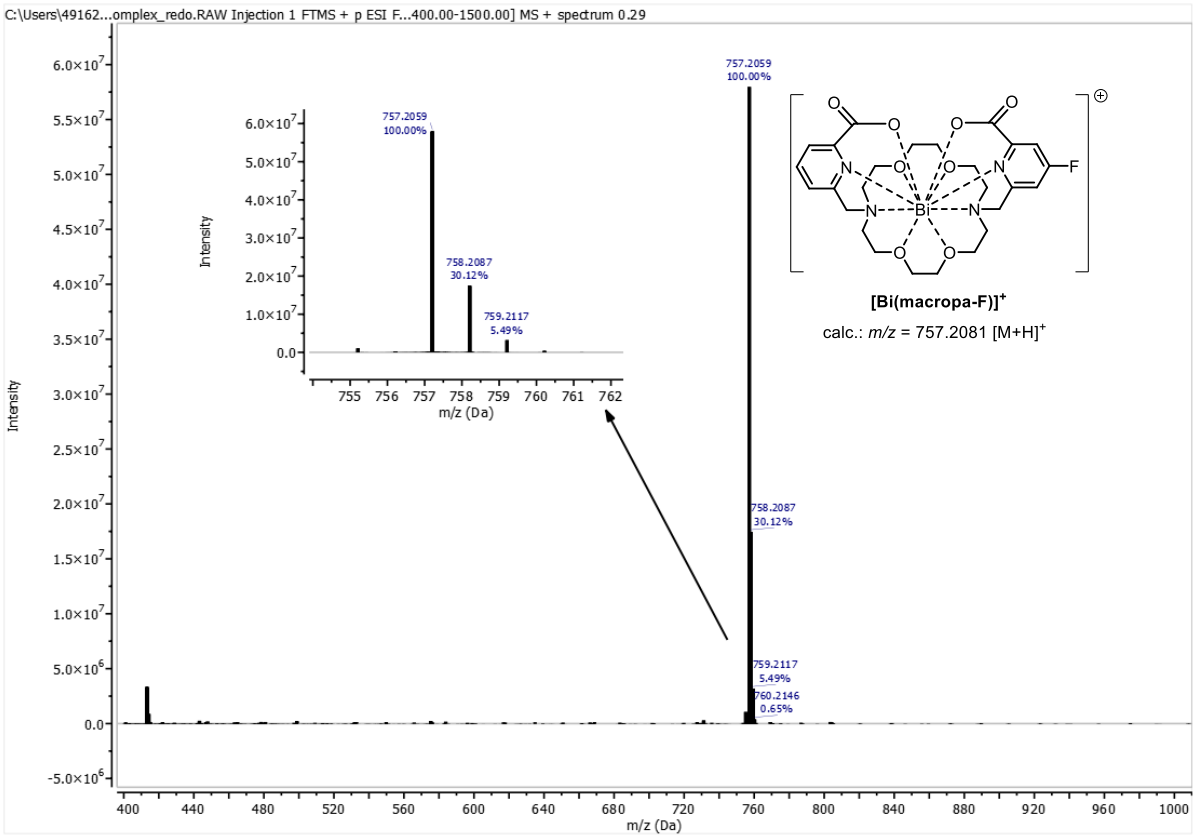


Figure S61: Positive ESI-MS of **[Bi(macropa-F)]$^+$** in methanol.

9.3 HPLC Chromatograms

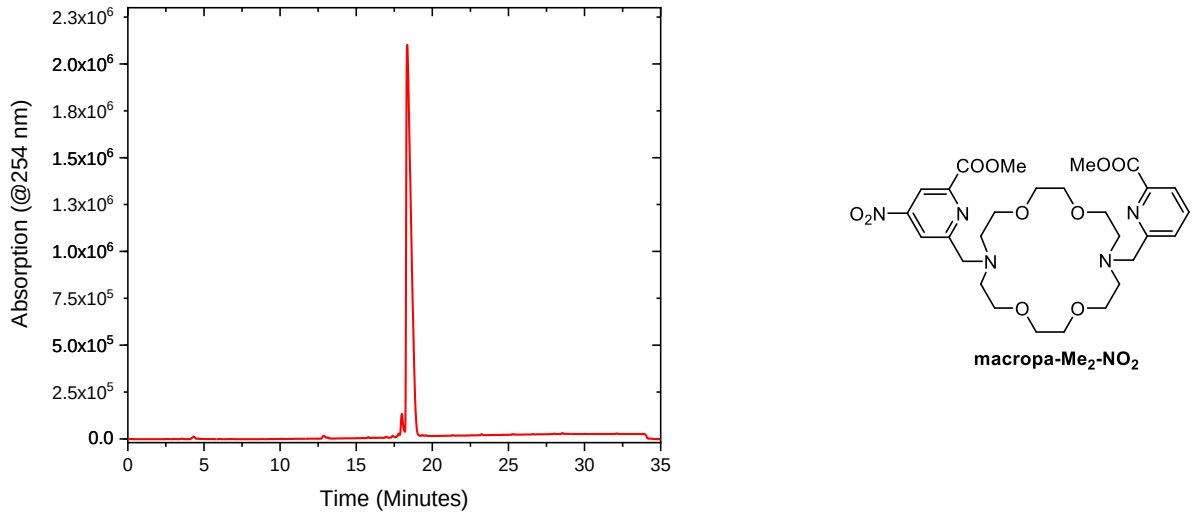


Figure S62: Analytical HPLC-chromatogram of **macropa-Me$_2$-NO$_2$** (HPLC method: *system 3*).

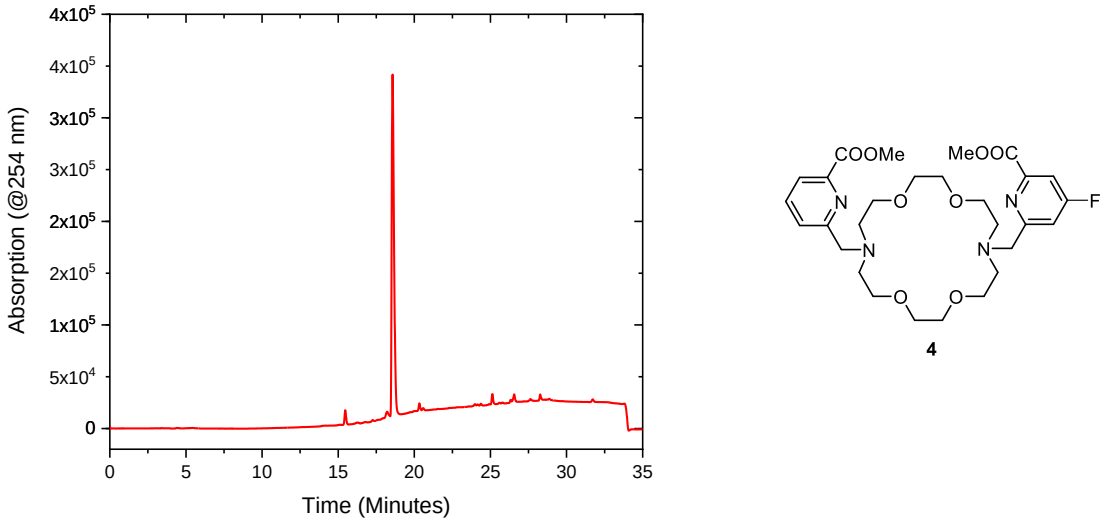


Figure S63: Analytical HPLC-chromatogram of **4** (HPLC method: *system 3*).

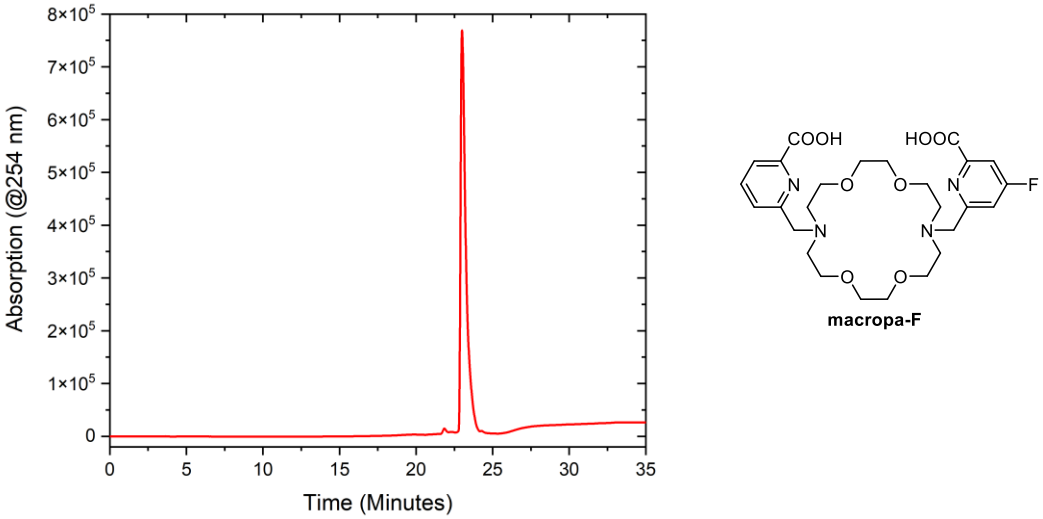


Figure S64: Analytical HPLC-chromatogram of **macropa-F** (HPLC method: *system 3*).

10. References

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