Supporting Information

Mechanistic Insights into Bismuth(III) Inhibition of SARS-CoV-2 Helicase

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Experimental Procedures

Cell lines and virus

VeroE6 (African green monkey kidney) and HEK293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) at 37℃. SARS-CoV-2 Omicron BA.5 and XBB strains were isolated from the respiratory tract of confirmed COVID-19 patients in Hong Kong. All experiments involving live SARS-CoV-2 followed the approved standard operating procedures of the Biosafety Level 3 facility at the Department of Microbiology, The University of Hong Kong.

Chemical and biochemical reagents

ATPase assay kit (ab234055, Abcam) was purchased from Abcam, BODIPY™ FL ATP-γ-S was from Invitrogen, cell proliferation kit (XTT) was purchased from Roche. SARS-CoV-2 NSP13 antibody (A20311, ProSci9183 and NBP-07055) were purchased from ABclonal, ProSci-inc and NOVUS respectively. SARS-CoV-2 NSP12 pAb(A20233) was also provided by ABclonal. GeneJuice® Transfection Reagent (70967) was purchased from Sigma-Aldrich. Immunoprecipitation kit with Protein A+G Magnetic Beads (P2179M) was purchased from Beyotime. Alexa Fluor 488(A11008)/594(R37121)-conjugated secondary antibodies and 4,6-diamidino-2-phenylindole (DAPI) (D3571) were purchased from Thermo Fisher Scientific. GelRed Nucleic acid stain (SCT123) was purchased from Sigma-Aldrich. $Bi(NAC)_3$ and $Bi(GSH)_3$ were freshly prepared by mixing colloidal bismuth citrate (CBS) with 3 molar equivalents of N-acetyl-L-cysteine(NAC) or glutathione(GSH) respectively, other bismuth compounds were synthesized by ourselves. All chemicals were from Thermo Fisher unless otherwise specified.

Overexpression and purification of SARS-CoV-2 nsp13

SARS-CoV-2 nsp13 was overexpressed and purified similarly as previously described.[1] Briefly, *E. coli* BL-21(DE3) contained pET-28a-nsp13 plasmid was cultured in LB medium with 50 μg/mL kanamycin overnight at 37 ℃ for 24 hours. The bacteria were then amplified to 4L fresh medium at a ratio of 1:200. When OD_{600} reached 0.6-0.8, the overexpression of nsp13 was induced by addition of 200 μM isopropyl β-d-1-thiogalactopyranoside (IPTG) at 16 °C for 18 hours with agitation at 200 rpm. Centrifuged with 6,000 g for 10 min to collect bacteria at 4 ℃. The pellets were suspended in lysis buffer (20 mM Tris, 500 mM NaCl, pH 7.4) with 0.1% Triton X-100 and Protease Inhibitor Cocktail, then sonicated. The bacterial lysate was centrifuged with 12,000 g at 4 °C for 10 min. The recombinant protein was purified

by a 5 mL Ni(II)-charged HiTrap® Chelating column (GE Life Sciences), and washed by the buffer with 250 mM imidazole. The eluted helicase was collected and checked by SDS-PAGE, and the purest fractions were pooled together and subjected to thrombin-digestion of His_6-tag, followed by removing His_6-tag and undigested protein via the 5 mL HiTrap® Chelating HP column. The protein was further purified by HiLoad® 16/600 Superdex® 200 prep grade column (GE Life Sciences).

ATPase assay

As previously described,[1] ATPase assay kit (ab234055, Abcam) was used to measure the release of ATP's phosphate. Briefly, to a 50 μL ATPase reaction buffer, 20 nM helicase was incubated with bismuth compounds at different concentrations for 10 min at 25 ℃ followed by the addition of 50 μL reaction buffer which contained 1 μL substrate to initiate the reaction for another 20 min. Finally, 15 μL reaction developer was added to per well for 30 min. The absorbance of mixture at 650 nm was measured by SpectraMax iD3 Multi-Mode microplate reader and the IC_{50} values were determined by nonlinear regression using GraphPad Prism. The assays were performed in triplicate.

FRET DNA duplex unwinding assay

The FRET-based DNA unwinding activity assay was performed based on a previously described method.[2] DNA oligos were prepared:

FL-Cy3 oligo (5′TTTTTTTTTTTTTTT TTTTCGAGCACCGCTGCGGCTGCACC(Cy3)-3′),

RL-BHQ oligo (5'-(BHQ2)GGTGCAGCCGCAGCGGTGCTCG-3′)

RL oligo (5′-GGTGCAGCCGCAGCGGTGCTCG-3′) (Metabion)

The DNA oligomers were synthesized and purified by HPLC. The two oligomers mixed at a ratio of FL-Cy3: RL-BHQ of 1:1.5 for the final concentrations of 10 μM and 15 μM, respectively were annealed in annealing buffer containing 20 mM Tris-HCl pH 8.0, 150 mM NaCl by heating to 90 °C for 2 min in thermocycler (S1000™ Thermal Cycler, Bio-Rad), then cooling slowly to 25 °C at the rate of ~1 °C/min. In the 96-well black polystyrene microplate, 20 nM helicase was incubated with metal compounds at different concentrations in 95 μL reaction buffer (20 mM Tris-HCl buffer, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 5 mM TCEP, 0.1 mg/ml BSA and 10% glycerol) for 10 min at 25 ℃. Then, 2 μL 100 mM ATP, oligomers (FL-Cy3, RL-BHQ mixture) were added to give rise to a final concentration of FL-Cy3: RL-BHQ oligo and RL oligo at 5 nM and 10 nM respectively. After incubation for 2 min, the changes in fluorescence were measured at excitation/emission wavelengths 550/620 nm by SpectraMax iD3 Multi-Mode microplate reader. The relative DNA unwinding activity was expressed as a percentage, *i.e.,* the

ratio between the activity of the samples in the presence of metal compounds and that of the control sample. IC_{50} values were determined by nonlinear regression using GraphPad Prism. The assays were performed in triplicate.

Cell viability assay

Cell proliferation assay (XTT, Roche) was performed to detect the cytotoxicity of the bismuth compounds. VeroE6 cells ($1x10^4$) were seeded in 96-well plates, then incubated with different concentrations of compounds for 48 hours. Next, all medium was removed and washed three time by PBS. XTT substrate was added with fresh medium at 37 ℃ for 4 hours, then OD_{495} was recorded by SpectraMax iD3 Multi-Mode microplate. Compounds which dissolved in DMSO were kept the final concentration of 1% DMSO. The CC_{50} values of the compounds were calculated by GraphPad Prism.

Viral load reduction assay

Viral load reduction assay was performed on the VeroE6 cells, as described previously.[3] Supernatants from the Omicron BA.5 strain infected cells (MOI= 0.01) were collected at 48 h.p.i. for qRT–PCR analysis of virus replication. In brief, the viral supernatant was lysed with 50 μL RLT buffer and then extracted for total RNA with the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany). Real-time one-step qRT–PCR was used for quantitation of SARS-CoV-2 viral load using the One Step TB Green® PrimeScript™ RT-PCR Kit II (Takara). The primers and probe sequences were against the RNA-dependent RNA polymerase/helicase (RdRP/Hel) gene region of SARS-CoV-2, as previously described.[4]

Plaque reduction assay

VeroE6 cells were seeded at $2x10^5$ per well in 24-well plate. After 24 hours incubation, infected cells with 50 plaque forming units (PFU) of SARS-CoV-2 Omicron BA.5, were treated with the compounds at different dose or without drugs as control. Monolayers were then overlaid with media containing 1% low melting agarose inverted and incubated for another 72 hours, the wells were fixed with 10% formaldehyde for 12 hours. After removal of the agarose plugs, the monolayers were stained with 0.7% crystal violet and plaques were counted. The inhibition percentage of plaque relative to control was calculated to confirm EC_{50}.[3]

Immunofluorescence staining

The antigen expression was detected after transfecting nsp12 and nsp13 plasmids in HEK293T cells with primary SARS-CoV-2 NSP13 antibody, SARS-CoV-2 NSP12 antibody and Alexa Fluor 488/594-

conjugated secondary antibodies. Cell nuclei were stained with the 4,6-diamidino-2-phenylindole (DAPI). The images were captured by the Confocal Imaging system, ZEISS LSM 980.

Cellular bismuth uptake

About 1x10^5 VeroE6 cells were seeded in 12-well plate. Next day, cells were treated with different compounds including CBS, $Bi(GSH)_3$ $Bi(Tro\text{-}NH_2)_3$, $Bi(6\text{-}TG)_3$ and Bi(TMPP) at 1, 2, 5, 10, 20, 50 and 100 μM for 24 hours, then washed by PBS for three times to remove the residual drugs. Cells lysis was collected with 1xRIPA (abcam,288006) and centrifuged with 3,000 g for 5 min at 4 ℃ to get supernatant. Next, all samples were diluted with 1% HNO_3 to a detectable concentration and analyzed by ICP-MS (Agilent 7700). ^{209}Bi contents were calculated by the bismuth standard curve (Sigma). The cellular bismuth level was further normalized by protein concentration each sample. The assays were performed in triplicate.

ATP-γ-S fluorescence assay

In a volume of 100 μL, 20, 50 and 100 nM nsp13 (helicase) and 10 nM BODIPY™ FL ATP-γ-S(Invitrogen) were added into reaction buffer (20 mM Tris, 500 mM NaCl, pH 7.4, 5 mM $MgCl_2$) respectively. Fluorescence signals were measured by SpectraMax iD3 Multi-Mode microplate reader at excitation/emission wavelengths 502/545 nm. For the drugs' inhibition assay, 100 nM nsp13 was incubated with different concentration of metal compounds in the reaction buffer for 10 min at 25℃, then 10 nM BODIPY™ FL ATP-γ-S was added. Fluorescence signals were recorded.

Electrophoretic Mobility Shift Assay (EMSA)

Electrophoretic Mobility Shift Assay (EMSA) was performed to detect the DNA binding capability of SARS-CoV-2 nsp13. 100 nM dsDNA was incubated with the increasing concentration of nsp13 (0, 0.5, 1, 2 and 5 μM) in 20 μL binding buffer (20 mM Tris pH 7.4, 20 mM NaCl, 1 mM TCEP, 5 mM $MgCl_2$) for 15 min at 37℃. For the drug treated assays, nsp13 incubated with a gradient amount of CBS for 2 hours at room temperature before the DNA-binding assay. The reaction was stopped by the addition of 4μL loading buffer (50% glycerol, 0.02% bromophenol blue). Then, 20 μL reaction mixture was loaded onto 8% native-polyacrylamide gels and electrophoresed in 1xTBE buffer at 150 V for 1 hour. Gels were stained by GelRed (Sigma Aldrich) for 10 min. The images were captured by iBright™ CL750 Imaging System (Thermo Fisher Scientific).

Antibody conjugation with metal-tags

Specific nsp12, nsp13 detection antibodies were labeled with ^{163}Dy and ^{165}Ho respectively by using the MaxPAR antibody conjugation kits according to the commercial protocol.

Magnetic beads functionalization with capture antibodies

To conjugate nsp12 and nsp13 capture antibodies on magnetic beads, N-hydroxy succinimide (NHS) activated magnetic beads were used to immobilize proteins through primary amines of proteins covalently. After removing preservation buffer using a magnetic stand, the ice-cold hydrochloric acid (1 mM, pH 3.0) was used to activate the NHS activated magnetic beads. Antibodies (50 μg) were added into 4 mg magnetic beads immediately after removing hydrochloric acid. The antibodies and beads mixture were incubated for 2 hours on the shakers at room temperature. After incubation, glycine solution (0.1 M, pH 2.0) was added, the production of antibodies labeled magnetic beads was washed with ultrapure water twice following by quenching the reaction in ethanolamine (3 M, pH 9.0). At last, the labeled magnetic beads were washed with ultrapure water and PBS twice and preserved in PBS.

***Cellullo* target enrichment approach based on magnetic-beads capture-detection system**

In brief, the magnetic beads modified with the nsp13 capture antibodies were blocked in the blocking buffer (0.1% PBS, 0.1% Tween-20 and 2.5% BSA) for 30 min at room temperature, followed by incubation with cell lysates (5-fold dilution) for 30 min at room temperature. The magnetic beads were washed with PBS containing 0.1% Tween-20 for three times and then incubated with ^{165}Ho-labeled nsp13 detection antibodies for 30 min at room temperature subsequently. The remaining unreacted detection antibodies were removed by washing with PBS four times. The reacted metal-labeled detection antibodies were released into solution by adding100 μL 1% HNO_3 as an elution buffer. The released production was diluted with 300 μL 1% HNO_3 and 5 ppb indium (^{115}In) as the internal standard for ^{165}Ho, ^{209}Bi, ^{66}Zn and ^{115}In quantification by ICP-MS.

Co-immunoprecipitation and quantification of the content of nsp12 by the lanthanide tagged antibody CO-IP

The Co-immunoprecipitation was performed using a commercial kit (Beyotime, P2179M) according to the protocol. In brief, nsp12 and nsp13 were transfected together into HEK293T cells, treated with 0, 10, 50 and 100 μM Bi(TMPP) (or the same concentration of $Bi(6\text{-}TG)_3$) for 24 hours. Cell lysate was collected with 1 mL lysis buffer (1% Triton-100X, proteases inhibitor in PBS) for 30 min at 4 ºC, centrifuged at 12,000 rpm for 10 min 4 ℃. The supernatants were collected for the CO-IP experiment. Protein A+G magnetic beads were mixed with antibody working solution, resuspended and incubated by inverting

mixer for 60 min at room temperature. The protein A+G magnetic beads were washed by gently blowing with a pipette using 500 μL TBS buffer three times then resuspended in TBS according to the original volume. Protein A+G magnetic beads bound to antibody was incubated with protein sample overnight at 4 °C. Next day, the supernatant was separated and removed. The magnetic beads were washed and resuspended with 500 μL lysis buffer gently for three times. 90 μL SDS-PAGE sample Loading Buffer (1x) was added to the magnetic beads, and heated at 95 °C for 5 minutes, the supernatant protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for Western Blot analysis. To determine the content of nsp12 that interacts with nsp13, a similar method to the target enriched approach was used. Briefly, the nsp13 antibody-labeled magnetic beads were blocked in the blocking buffer (0.1% PBS, 0.1% Tween-20 and 2.5% BSA) for 30 min at room temperature, followed by incubation with cell lysates (5-fold dilution) for 30 min at room temperature. The magnetic beads were washed with PBS containing 0.1% Tween-20 for three times and then incubated with ^{163}Dy-labeled nsp12 detection antibodies for 30 min at room temperature subsequently. The remaining unreacted detection antibodies were removed by washing with PBS four times. The reacted metal-labeled detection antibodies were released into solution by adding 100 μL 1% HNO_3 as elution buffer. The released production was diluted with 300 μL 1% HNO_3 and 5 ppb indium (^{115}In) as the internal standard for ^{163}Dy quantification by ICP-MS.

Dissociation constant (K_d) measurement

Dissociation constants were obtained by electronic spectroscopy. Briefly, Bi(III) complexes of interested were titrated in various thiol solutions and the absorbance at ~340 nm assigned to LMCT (Bi-S) transfer were monitored. The calibration curves of different bismuth thiolate complexes were employed to calculate the concentration of Bi(III) thiolate complexes after each addition. The dissociation constant (K_d=1/K) for [Bi(thiolate)$_n$] was determined by the measurement of free and bound thiolates as we did previously.[5]

Synthesis of metal compounds

5-nitroso-tropolone. Tropolone (2.5 g, 20.5 mmol) was dissolved in water (10 mL) and acetic acid (6 mL) solution. To the solution, sodium nitrite (1.7 g, 24.5 mmol) in 5 mL of water solution was added to the reaction mixture. The reaction solution was then stirred at 25 °C for 3 hours. The resulting brown solid was collected by vacuum filtration and washed with 50 mL of cool water to yield 5-nitroso-tropolone

(68% yield). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.85 (d, J = 40.0 Hz, 2H), 7.58 (dd, J = 12.2, 1.5 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d6): δ 185.7, 184.2, 152.3, 139.7, 130.3, 128.1, 124.2. ESI-MS (-) m/z 150.12, found for C7H4NO3 $[M-H]^-$ (calcd: 151.12).

5-amino-tropolone. 5-nitroso-tropolone (2.2 g, 14.6 mmol) was dissolved in 75 mL of ethanol. 5% palladium 10 on carbon (wetted with ca. 55% water, 0.3 g) was then added. A hydrogen balloon was placed on top and stirred overnight. The reaction mixture was filtered over celite and the filtrate evaporated to dryness. The dark brown solid was then dissolved in minimum amount of methanol and recrystallized with the addition of diethyl ether, the resulting golden precipitate was filtered and collected (1.45 g, 73% yield). ^{1}H NMR (400 MHz, acetone-d_6), δ (ppm): 7.14 (d, J = 12.1 Hz, 2H), 6.85 (d, J = 12.1 Hz, 2H), 5.69 (s, 2H). ^{13}C NMR (200 MHz, DMSO-d_6), δ (ppm): 185.7, 184.2, 161.1, 139.7, 156.5, 120.1, 124.2. ESI-MS (-) m/z 136.11, found for C7H6NO2 $[MH]^-$ (calcd: 136.13).

Bi(Tro-NH_2)$_3$. 5-amino-tropolone (0.3 g, 3.65 mmol) was dissolved in 40 mL MeOH, bismuth nitrate pentahydrate (0.59 g, 1.22 mmol) was then added. The white suspended mixture was then heated to 60 °C for 4 hours. The solution was rotor evaporated to half the original volume and 40 mL of water was then added to the mixture, the resulting brown precipitate was filtered and collected to give Bi(Tro-NH_2)$_3$ complex (0.75 g, 65% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 7.21 (d, J = 11.6 Hz, 2H), 6.72 (d, J = 12 Hz, 2H), 6.79 (s, 2H). ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 121.9, 135.7, 154.8, 170.0. HR-ESI-MS (+) m/z 481.0576, found for C14H12N2O4Bi$^+$ $[Bi(Tro\text{-}NH_2)_2]^+$ $[M]^+$ (calcd: 481.0595). TGA analysis: 63.84% weight loss at 500°C in compress air, proposed structure: Bi(Tro-NH_2)$_3$·H_2O.

Bi(Hino)$_3$. Hinokitiol (0.35 g, 2.13 mmol) was dissolved in 40 mL of 5:1 ratio Acetone: Water, bismuth nitrate pentahydrate (0.35 g, 0.71 mmol) was then added. The white suspended mixture was then heated to 80 °C for overnight. The solution was rotor evaporated until precipitate occurred, the mixture was then cooled to 0°C in an ice-bath to yield a brown-yellow solid. The precipitate was filtered and recrystallized in ethanol to give pure Bi(Hino)$_3$ complex (0.2 g, 41% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.36-7.40 (t, J = 8.4 Hz, 2H), 7.16-7.18 (d, J = 8.8 Hz, 2H), 6.9 (d, J = 8 Hz, 2H), 2.83-2.88 (hept, J = 8.8 Hz, 1H), 1.23 (d, J = 5.6 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 24.2, 39.8, 128.0, 129.0, 130.3, 137. 8, 160.6, 178.9, 179.5. ^{1}H NMR for ligand (400 MHz, $CDCl_3$), δ (ppm): 7.3-7.33 (dd, J = 3.6 Hz, 0.8 Hz, 2H), 7.2-7.23 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 8 Hz, 2H), 2.83-2.88 (hept, J = 8.8 Hz, 1H), 1.23 (d, J = 5.6 Hz, 6H). HR-ESI-MS (+) m/z 1419.4102, found for $[2M+Na]^+$ (calcd: 1419.4), m/z 721.2003 found for C30H33O6Bi $[M+Na]^+$ (calcd: 721.1973), m/z 535.1337, found for $[C20H22O4Bi]^+$ (calcd:

535.1316).

Bi(NTA). This complex was synthesized based on a previously reported method.[6] Nitrilotriacetic acid (0.215 g, 1.122 mmol) was dissolved in 60 mL water. The solution was heated to 70 °C. Bismuth subcarbonate, $(BiO)_2CO_3$ (0.286 g, 0.561 mmol) was then added. The mixture was then heated overnight and most of the solid dissolved. The mixture was filtered while hot, the filtrate was put into 4°C. freezer for 2 hours, the crystal filtered and collected, which gave Bi(NTA) as Bi(NTA) dihydrate (0.18 g, 36.5%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 4.03 (s, 6H). ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 63.0, 175.4. ^{1}H NMR for the ligand (400 MHz, DMSO-d_6), δ (ppm): 3.50 (s, 6H). HR-ESI-MS (+) m/z 398.0075, found for C6H6O6NBi $[M+H]^+$ (calcd: 398.0077).

Bi(TS)$_3$. This complex was synthesized based on a previously reported method.[7] 2-mercaptobenzoic acid (2.53 g, 1.67 mmol) was suspended in 50 mL ethanol, 7 mL of 25-30% ammonia was the added giving complete dissolution. Bismuth subcarbonate, $(BiO)_2CO_3$ (1.7 g, 0.835 mmol) was then added to the yellow solution. The mixture was then heated for 4 hours. The mixture was filtered while hot, the filtrate stand at room temperature overnight, the resulting crystal was filtered and collected, which gave yellow colored $Bi(TS)_3$ as $(NH_4)_3$Bi(thiosalicylate)$_3$ dihydrate (4.21 g, 67%). ^{1}H NMR (400 MHz, D_2O), δ (ppm): 7.32-7.34 (d, J = 7.2 Hz, 1H), 7.2-7.24 (dd, J = 7.6 Hz, 1H), 7.0 (dd+d, J = 8 Hz, 2H), ^{13}C NMR (400 MHz, D_2O), δ (ppm): 16.8, 57.4, 126.8, 126.8, 128.1, 131.5, 137.4, 143.4, 177.1. HR-ESI-MS (-) m/z 512.9509, found for $[C14H8O4Bi]^-$ (calcd: 512.9673). TGA analysis: 68.21% weight loss at 500°C in compress air, proposed structure: $Bi(TS)_3 \cdot 2H_2O$.

Bi(Tio)$_2$. Tiopronin (0.5 g, 3.06 mmol) was dissolved in 40 mL water, bismuth nitrate pentahydrate (0.495 g, 1.02 mmol) was then added. The solution instantly turned yellow. The yellow solution was stirred at room temperature for around 2 hours until all bismuth nitrate pentahydrate dissolved. The solution was rotor evaporated to dryness and minimum amount of methanol was used to dissolve it, excess diethyl ether was then added to precipitate it out as yellow solid. The yellow solid was then redissolved in minimum amount of water, 3 equivalent of sodium bicarbonate was added, and the solution was stirred for 30 minutes, the mixture was then added with excess ethanol giving light yellow precipitate. The light-yellow solid was filtered, washed with small amount of methanol and then diethyl ether and collected to give $Bi(Tio)_2$ complex (0.690 g, 32.4% yield). ^{1}H NMR (400 MHz, D_2O), δ (ppm): 3.8 (d, J = 6.8 Hz, 1H), 3.76-3.78 (d, J = 6.4 Hz, 2H), 3.56-3.62 (q, J = 7.2 Hz, 1H), ^{13}C NMR (400 MHz, D_2O), δ (ppm): 16.9, 23.8, 40.5 40.5, 43.6, 57.5, 176.1, 179.7, HR-ESI-MS (-) m/z 531.0027, found for

C10H15O6N2S2Bi $[M-H]^-$ (calcd: 531.0103).

Bi(bpy)$_2$. This complex was synthesized based on a previously reported method.[8] 2,2'-Bipyridine (0.5 g, 3.2 mmol) was dissolved in minimum amount of DMSO, bismuth triflate (1.05 g, 1.6 mmol) was then added. The suspension was then heated to 60 °C for around 6 hours until all bismuth triflate dissolved. Excess acetone was then added, the mixture was then cooled to 0°C in refrigerator for 4 hours, the solid formed was then filtered, washed with acetone and then diethyl ether and collected to give $Bi(bpy)_2(OTf)_3$ complex (0.376 g, 24.3% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): δ: 8.7 (d, J = 4.4 Hz, 1H), 8.39 (d, J = 8 Hz, 1H), 7.9 (dt, J = 7.6 Hz, 1.6Hz, 1H), 7.45 (dt, J = 4.8 Hz, 1.6Hz, 1H), ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 40.8, 116.2, 119.4, 122.6, 125.8. TGA analysis: 31.42% weight loss at 650°C in N_2, residual as Bi(III) triflate, proposed structure: $Bi(bpy)_2\cdot(triflate)_3$.

Bi(6-TG)$_3$·OTf. Bismuth triflate (0.3 g, 0.457 mmol) was added to 5 mL ethanol with the help of a few drops of Trifluoromethanesulfonic acid (CF_3SO_3H) to dissolve and then the solution was added dropwise into 75 mL ethanol solution of 6-thioguanine(6-TG) (0.23 g, 0.137 mmol). The mixture was refluxed at 80 °C for 3 hours until yellow precipitate formed. The precipitate was then filtered, washed with acetone and then diethyl ether and collected to give the adduct Bi(6-TG)★OTf as $[Bi(6\text{-}TG)_3(OTf)_2]$★OTf as final product (0.218 g, 41.2% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 12.7 (bs, 1H), 12.0 (s, 1H), 8.0 (s, 1H), 6.5-6.7 (s, 2H). ^{1}H NMR for ligand (400 MHz, DMSO-d_6), δ (ppm): 12.6-12.9 (s, 1H, exchange proton), 12.2 (s, 1H), 8.0 (s, 1H, exchange proton), 6.5 (s, 2H). ^{13}C NMR (400 MHz, DMSO-d_6 with few drops of 37.5% DCl in D_2O), δ (ppm): 27.0, 48.9, 72.3, 118.7, 139.4, 146.3, 154.9, 171.6. TGA analysis: 80.82% weight loss at 500°C in compress air, proposed structure: $[Bi(6\text{-}TG)_3(OTf)_2]$★OTf.

Bi(6-TG)$_3$. This complex was synthesized based on a previously reported method.[9] 6-TG (0.5 g, 3 mmol) was added to 20 mL water, sodium hydroxide (0.36 g, 9 mmol) was then added to turn the thioketone to thiolate formed by stirring for 30 min in above basic solution until all 6-TG dissolved. Bismuth nitrate pentahydrate (0.484 g, 1 mmol) was then added, the solution had yellow precipitate, the solution was stirred for 3 hours until all the white colored Bismuth nitrate pentahydrate dissolved, the precipitate was then filtered, washed with methanol and then collected to give the Bismuth thiolate complex as final product (0.53 g, 74% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 12.41 (s, 1H), 7.82 (s, 1H), 6.28 (bs, 2H). ^{13}C NMR (400 MHz, DMSO-d_6 with few drops of 37.5% DCl in D_2O), δ (ppm): 27.0, 48.9, 72.3, 118.7, 139.4, 146.3, 154.9, 171.6. ^{1}H NMR for ligand (400 MHz, DMSO-d_6), δ (ppm): 12.6-12.9

(s, 1H, exchange proton), 12.2 (s, 1H), 8.0 (s, 1H, exchange proton), 6.5 (s, 2H). TGA analysis: 80.86% weight loss at 500°C in compress air, proposed structure: [Bi(6-TG)$_3$].

Bi(TMPP). This complex was synthesized according to our previously reported method,[10] the Bi(III) complexes of tetra (4-methylphenyl) porphyrin (TMPP) was synthesized by a generic method. 0.064 g tetra (4-methylphenyl) porphyrin (TMPP) was dissolved in pyridine (50 mL) and reflux for 1 hour. Bi $(NO_3)_3 \cdot 5H_2O$ (0.81g) was added and the mixture continued to be refluxed for 5 hours. Then the additional $Bi(NO_3)_3 \cdot 5H_2O$ (0.49 g) was added and the solution was refluxed overnight. Green products were collected and re-dissolved in chloroform after all the pyridine was removed in vacuum. The compound was purified by washing the column firstly with chloroform and then chloroform/methanol in the ratio of 8:1. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.85 (s, 8H), 8.26 (dd, 8H, J= 7.43, 1.90 Hz), 7.79 (m, 8H), 7.23 (m, 4H). ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 22.1, 124.0, 128.0, 128.3, 133.4, 135.1, 128.6, 138.7, 148.9. HR-ESI-MS (+) m/z 877.2717, found for $C48H38N4Bi^+$ $[M]^+$ (calcd: 877.2738).

Supplementary Figures

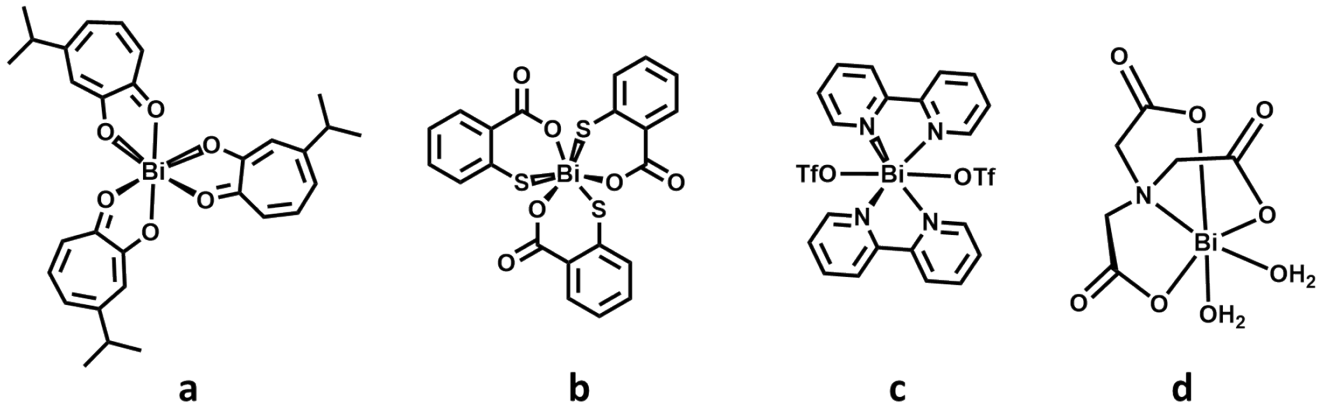


Figure S1. (a-d) Structures of selected bismuth compounds used in this study, (a) $Bi(Hino)_3$ (b) $Bi(TS)_3$ (c) $Bi(bpy)_2$ and (d) Bi(NTA).

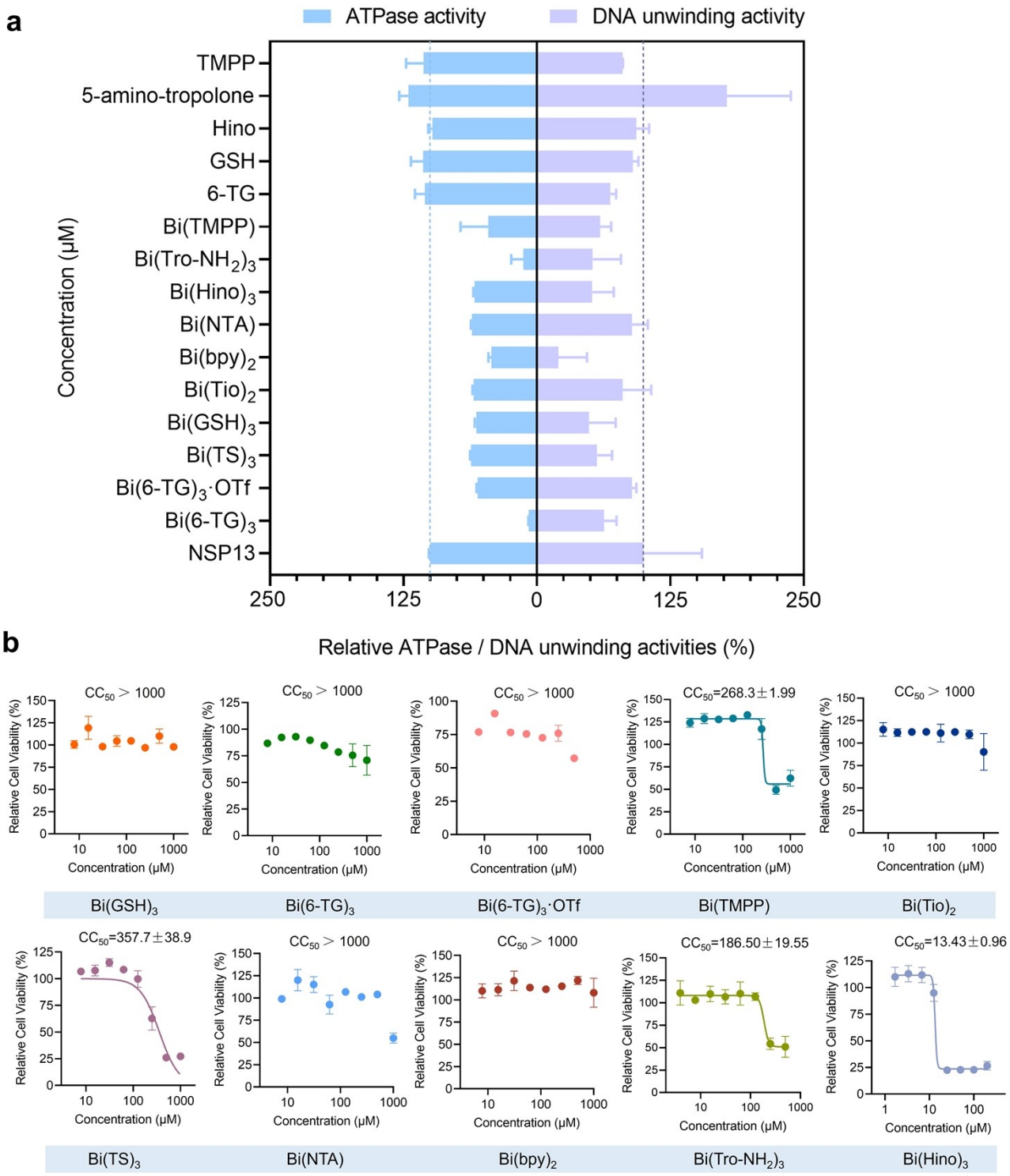


Figure S2. (a) Primary screening of bismuth compounds and corresponding ligands on their ability to inhibit ATPase and DNA unwinding activities of nsp13. (b) Half cytotoxicity (CC_{50}) of bismuth compounds against VeroE6 cells.

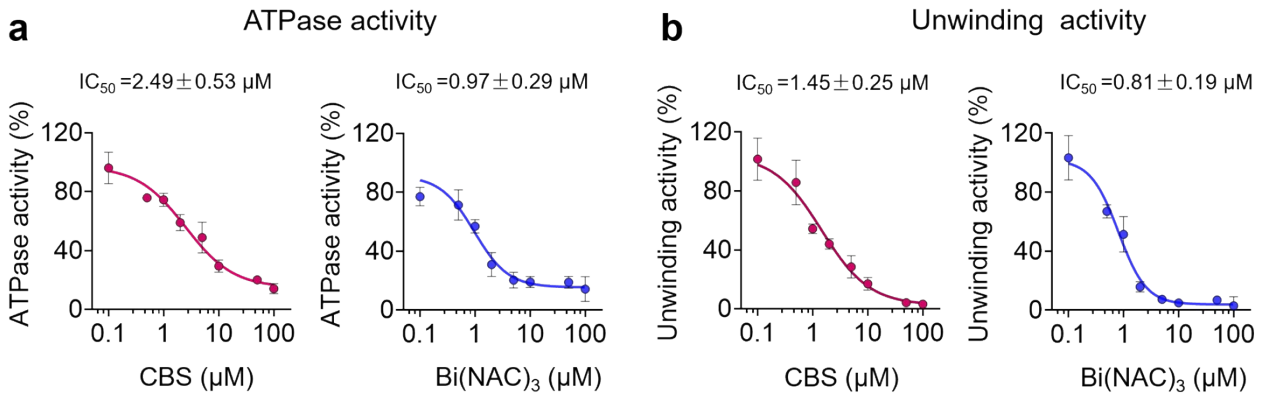


Figure S3. (a) The ATPase activity of CBS and $Bi(NAC)_3$. (b) The unwinding activity of CBS and $Bi(NAC)_3$.

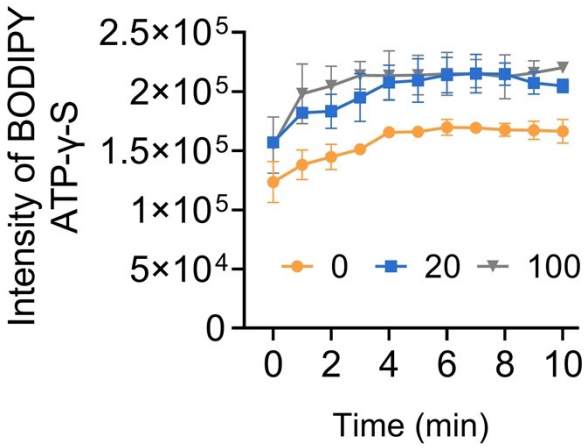


Figure S4. The fluorescence intensity of BODIPY ATP-γ-S upon the addition of nsp13 at different concentrations.

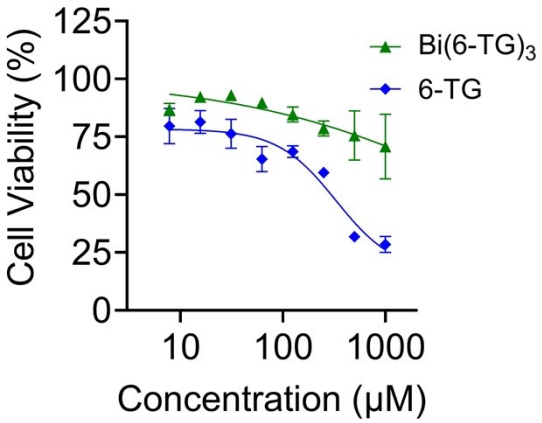


Figure S5. The CC_{50} values of $Bi(6\text{-}TG)_3$ and 6-TG in VeroE6 cells are >1000 μM and 331.2±21.3 μM respectively.

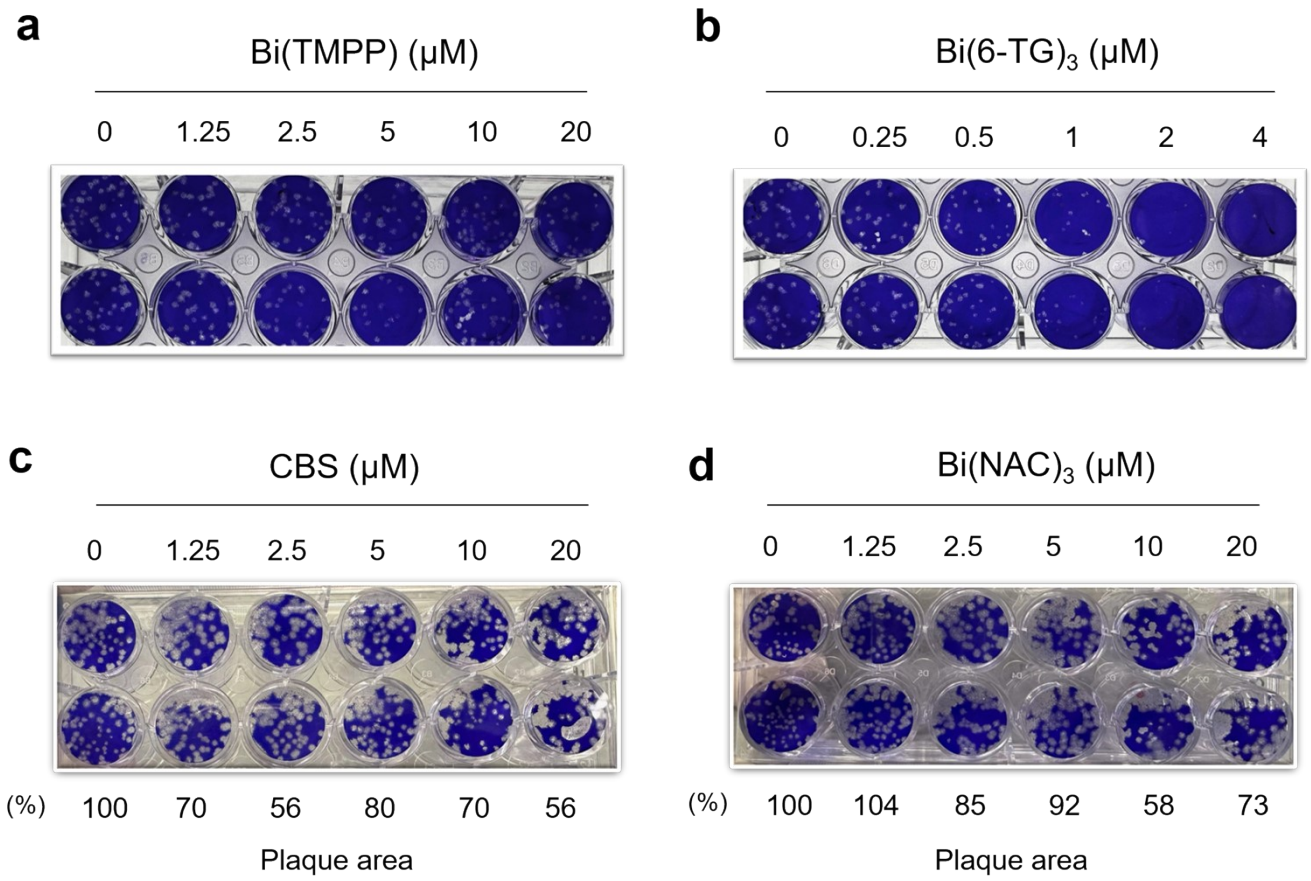


Figure S6. Plaque reduction assay for evaluation the antiviral activity of Bi(TMPP), $Bi(6\text{-}TG)_3$, CBS and $Bi(NAC)_3$ against SARS-CoV-2 Omicron BA.5.

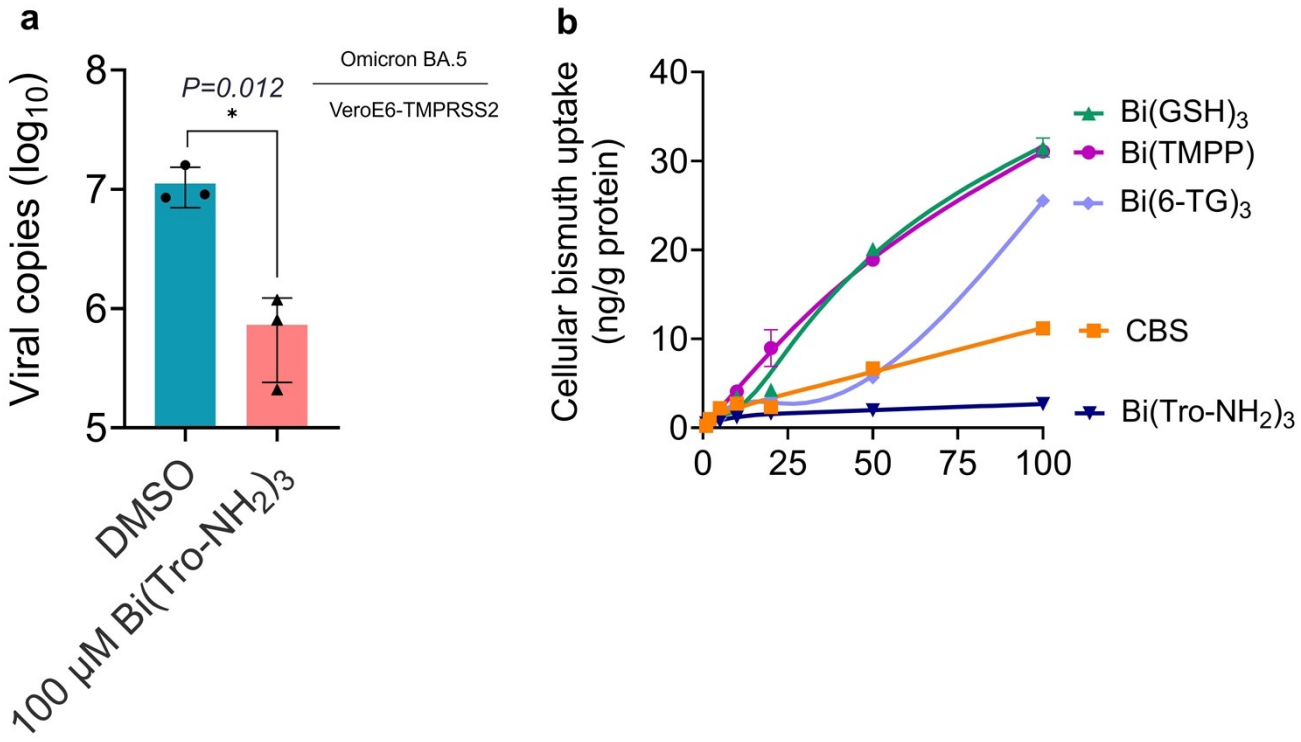


Figure S7. Antiviral activity and cellular bismuth(III) uptake. (a) Viral loads in cell culture supernatant quantified by reverse transcription (RT-qPCR). (b) Bismuth uptake in a dose dependent manner. VeroE6 cells were treated with CBS, $Bi(GSH)_3$, $Bi(6\text{-}TG)_3$, Bi(TMPP) and $Bi(Tro\text{-}NH_2)_3$ for 24 hours.

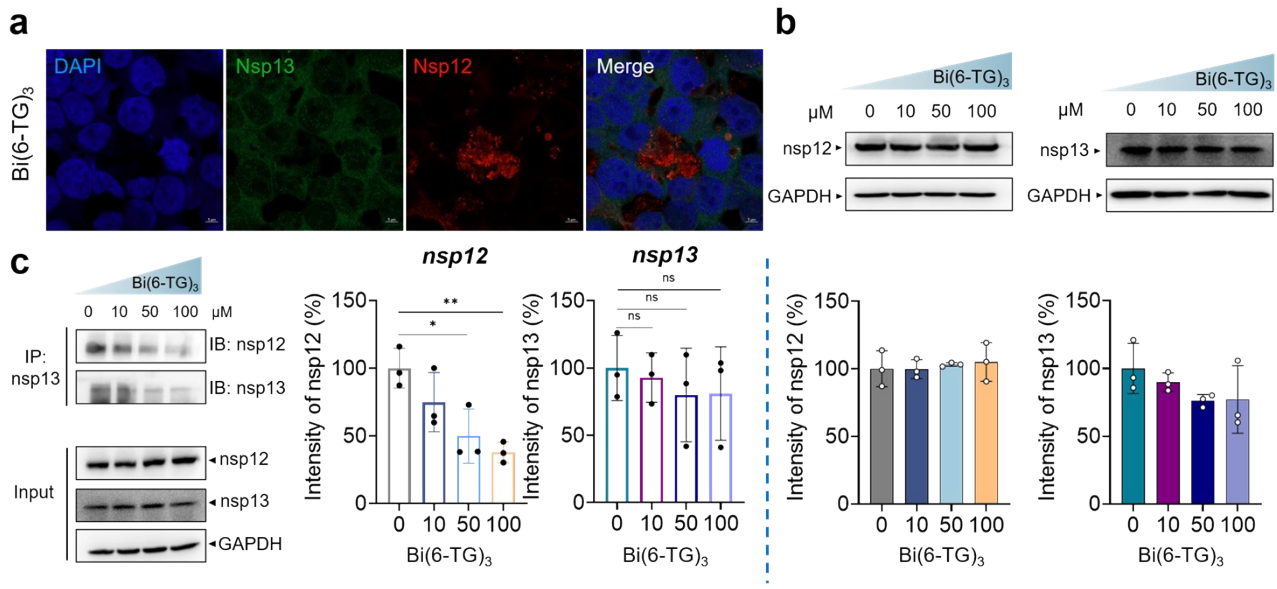


Figure S8 Bismuth disturbs the nsp13-nsp12 interaction. (a) Immunofluorescence staining images show the nsp13-nsp12 interaction before and after treating 50 μM $Bi(6\text{-}TG)_3$. The SARS-CoV-2 nsp12 and nsp13 antigens were stained in red and green respectively, while cell nuclei were stained in blue by DAPI (scale bar=5 μm). (B) Western blotting images of nsp12 and nsp13 expression after treating different concentrations bismuth compound, $Bi(6\text{-}TG)_3$. (C) Western blotting results for the CO-IP. Data are shown as mean ± SD. The statistical significance was calculated by unpaired two-tailed Student's *t*-test. **P*<0.05, ***P*<0.01, ****P*<0.001.

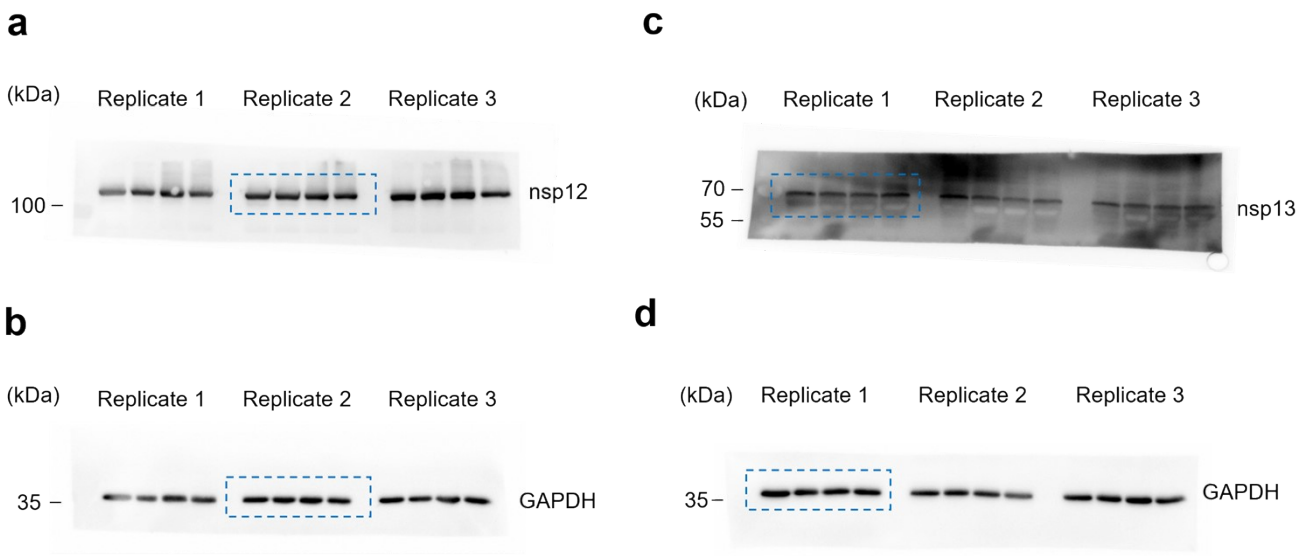


Figure S9. Protein expression of nsp12 and nsp13 (presented in Fig.6B). (a-b) nsp12 and corresponding internal standard (GAPDH) in HEK293T upon different treatment conditions. (c-d) nsp13 and corresponding internal standard (GAPDH) in HEK293T upon different treatment conditions.

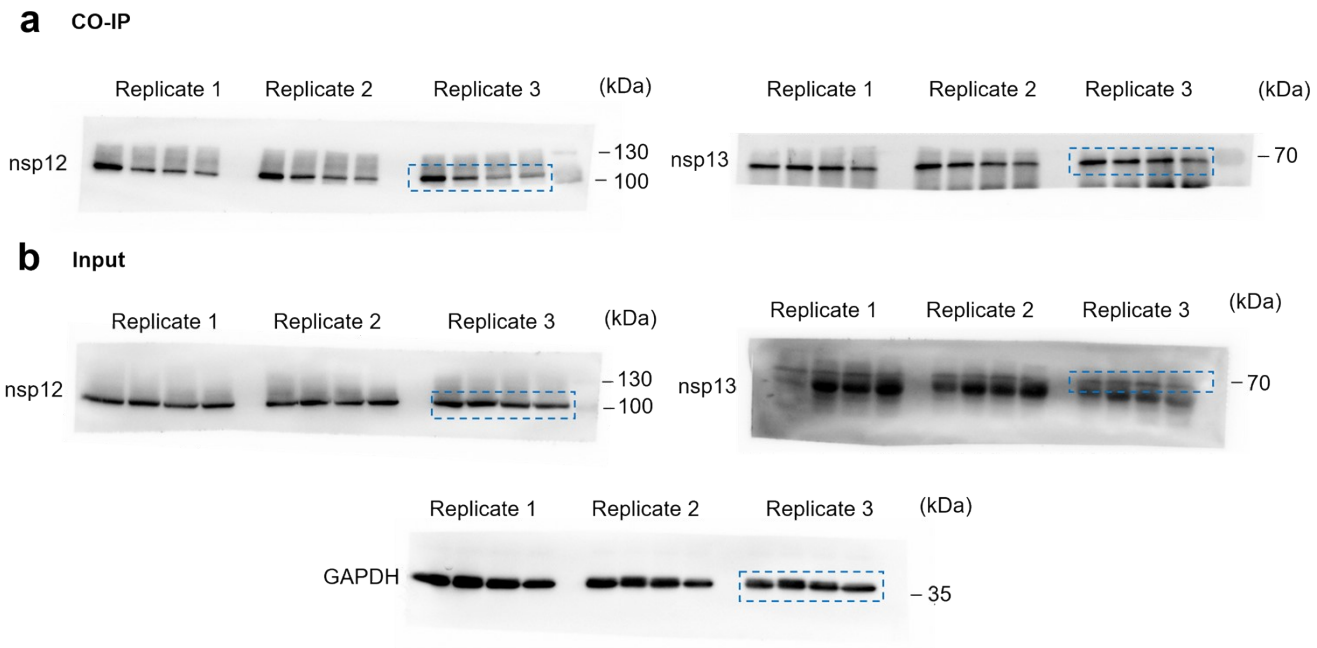


Figure S10. CO-IP results (presented in Figure 6C). (a) nsp12 and nsp13 expression after CO-IP. (b) Input of nsp12 and nsp13 expression.

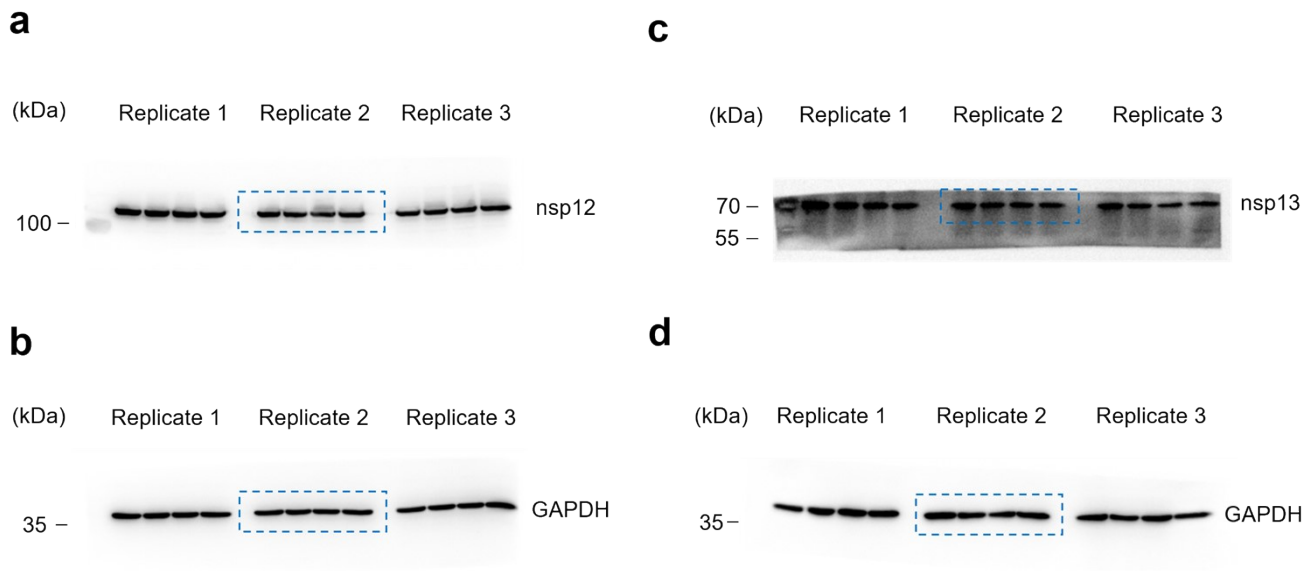


Figure S11. Expression of nsp12 and nsp13 (presented in Fig. S8b). (a-b) nsp12 and corresponding an internal standard (GAPDH) in HEK293T upon different treatment conditions. (c-d) nsp13 and corresponding an internal standard (GAPDH) in HEK293T upon different treatment conditions.

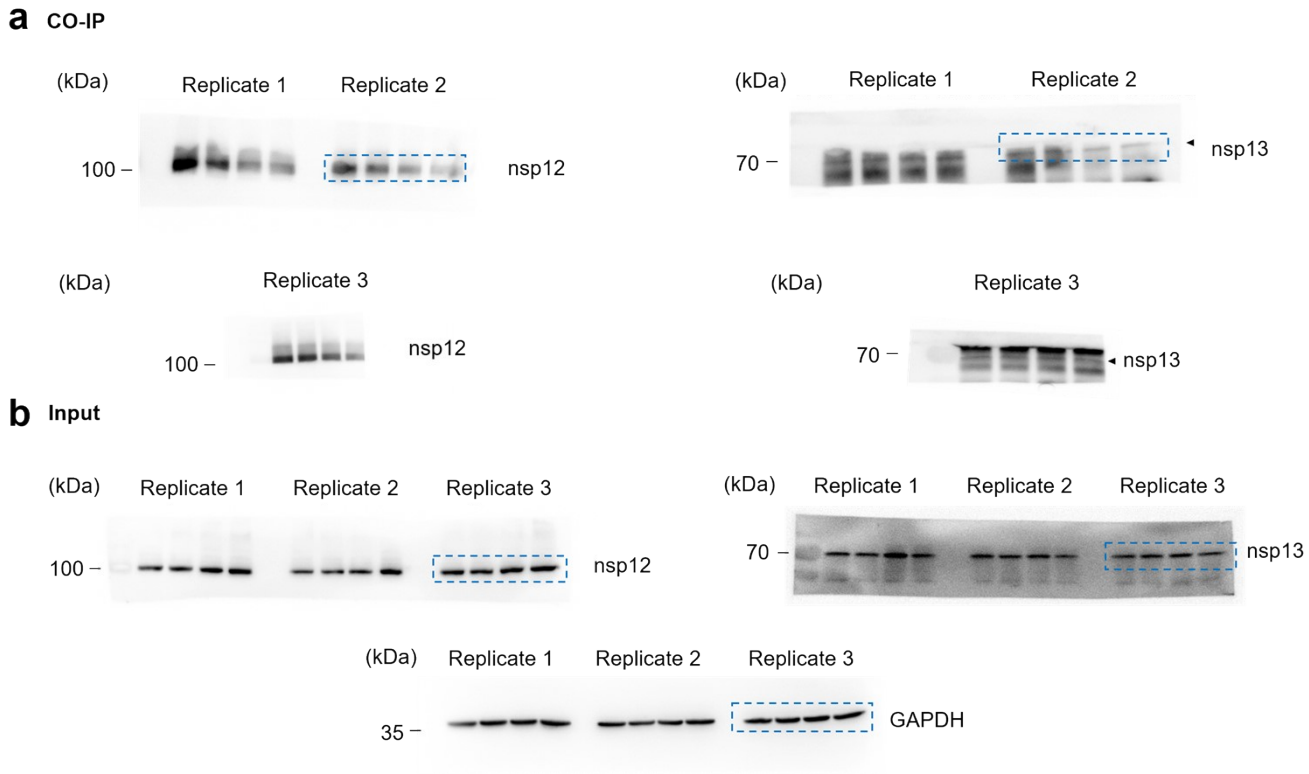
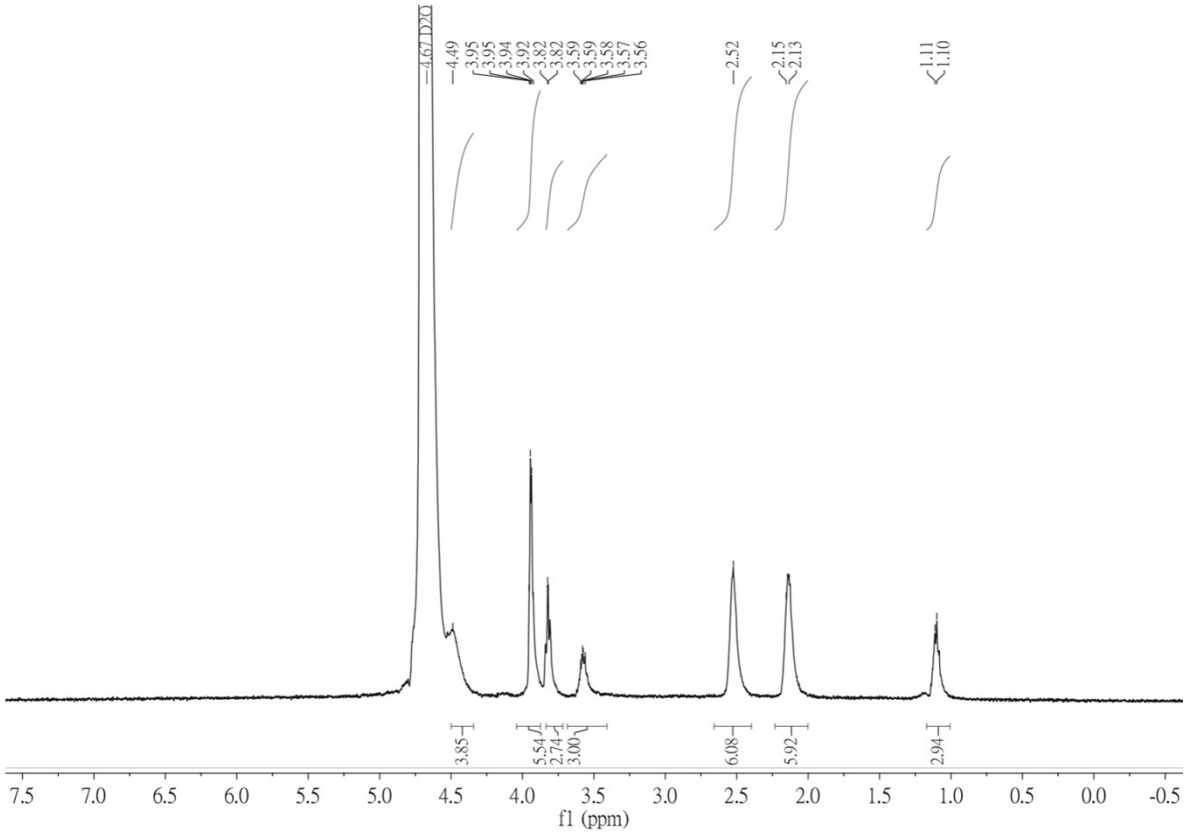


Figure S12. CO-IP results of Fig. S8c). (a) nsp12 and nsp13 expression after CO-IP. (b) Input of nsp12 and nsp13 expression.

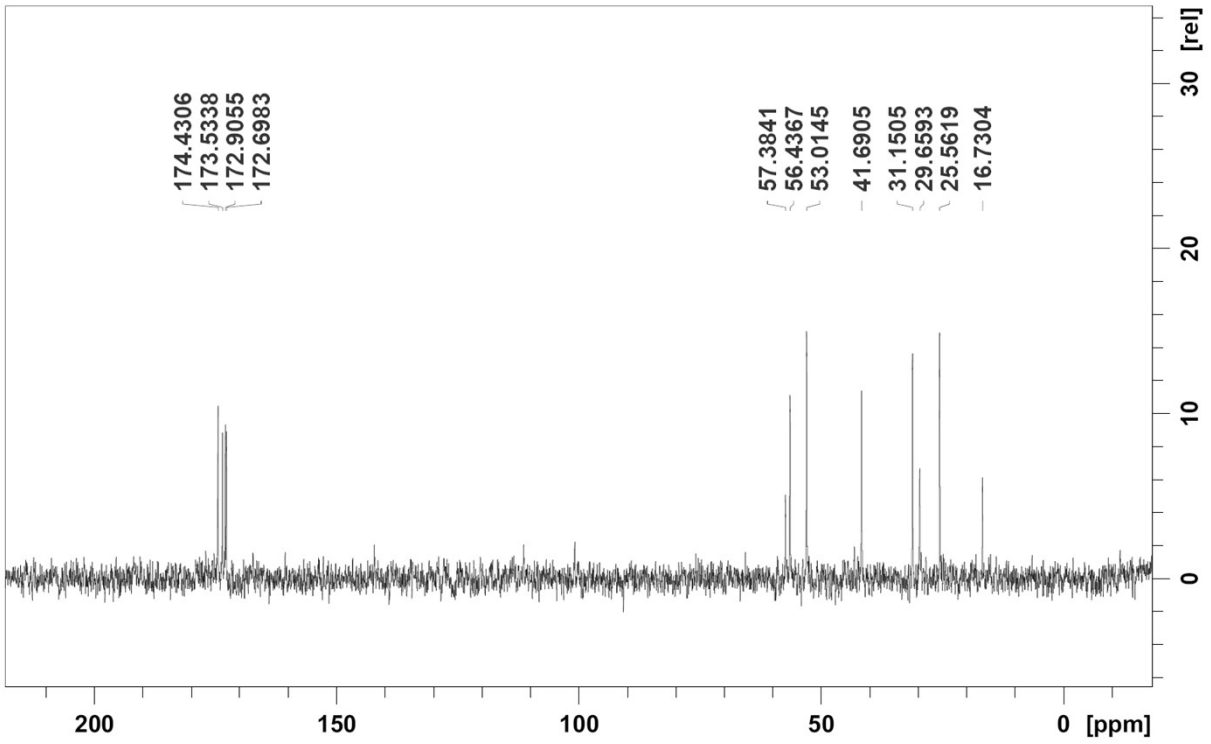
NMR Spectra

$Bi(GSH)_3$

1. 400 MHz ^1H NMR in D_2O

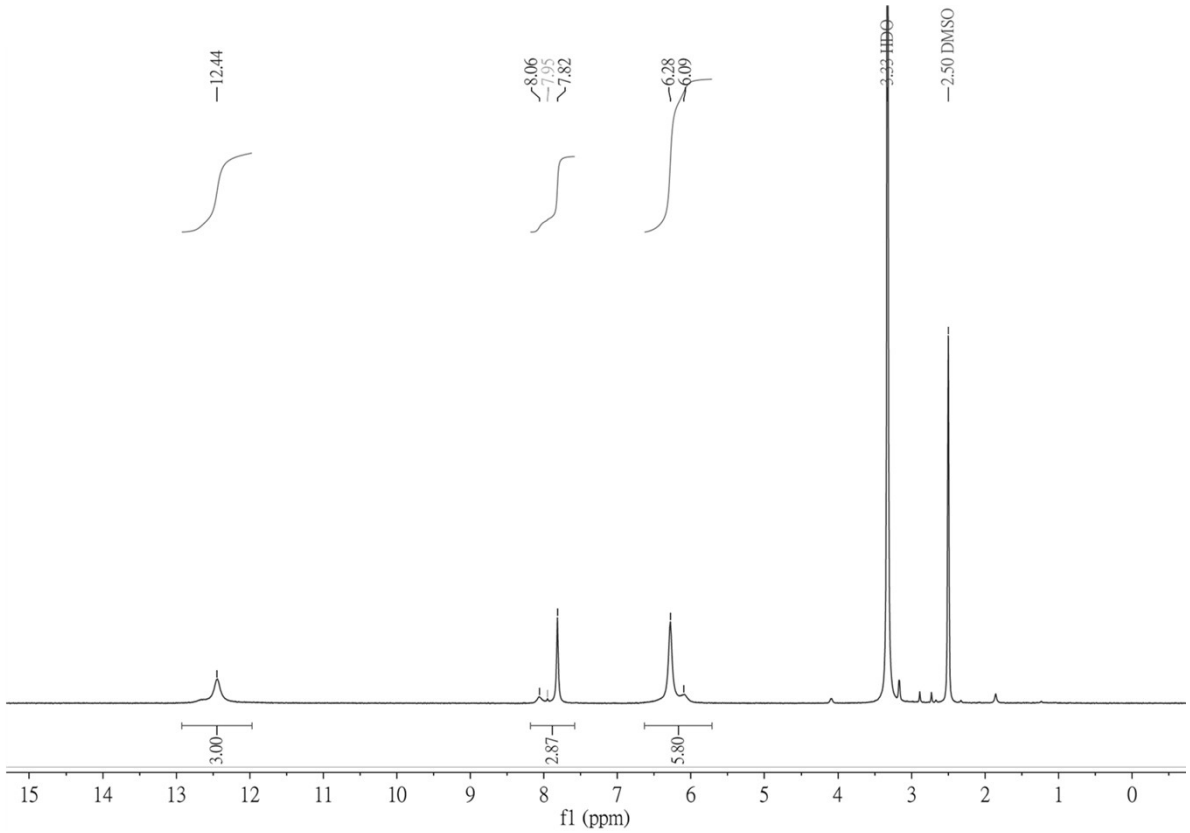


2. 100 MHz ^{13}C NMR in D_2O

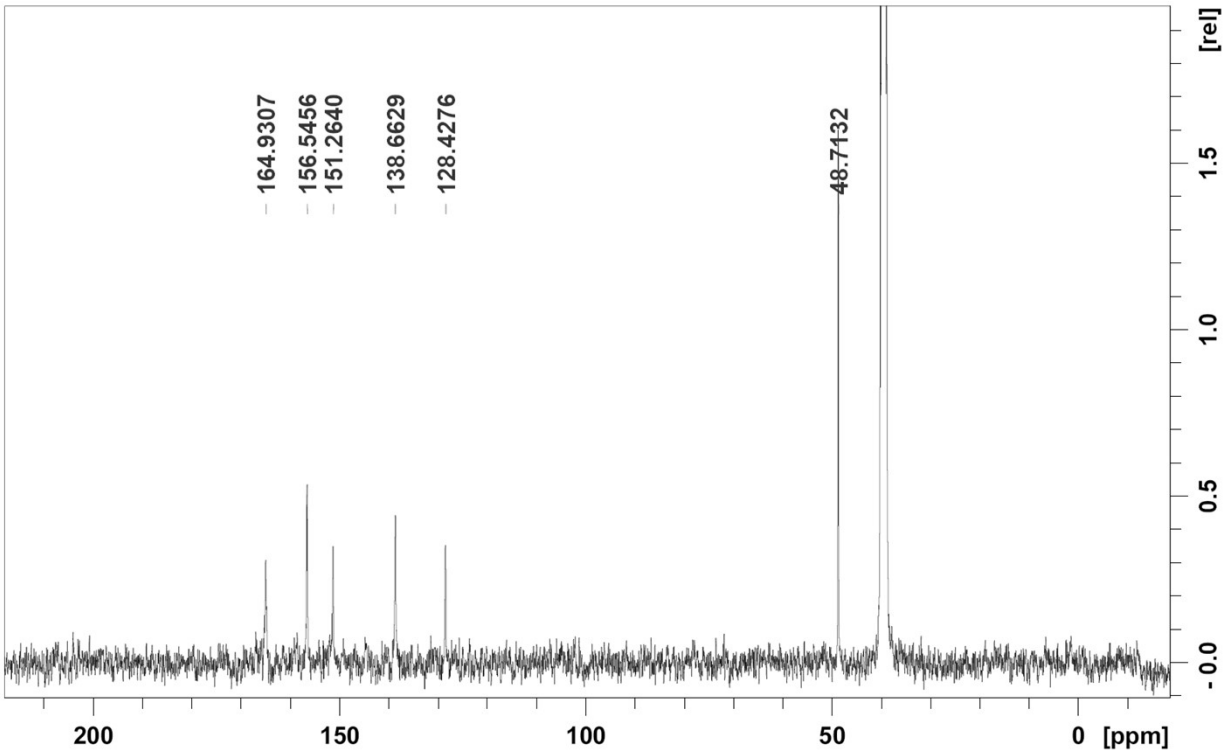


Bi(6-TG)$_3$

1. 400 MHz ^{1}H NMR in DMSO-d$_6$

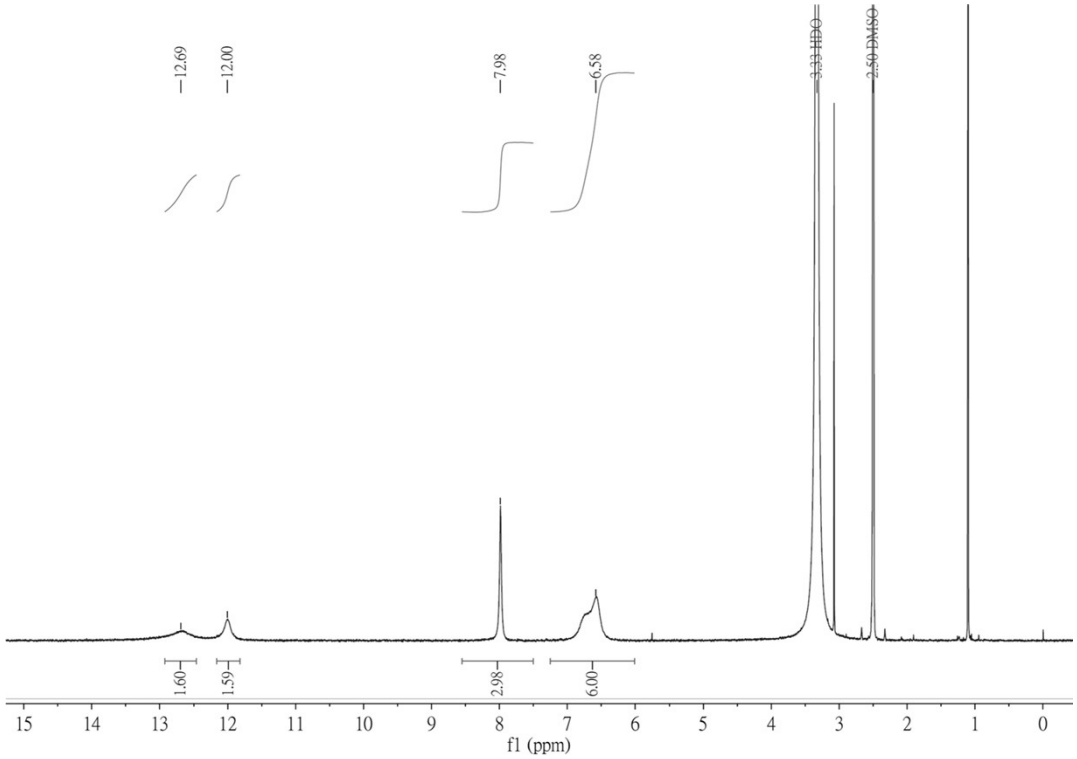


2. 100 MHz ^{13}C NMR in DMSO-d$_6$

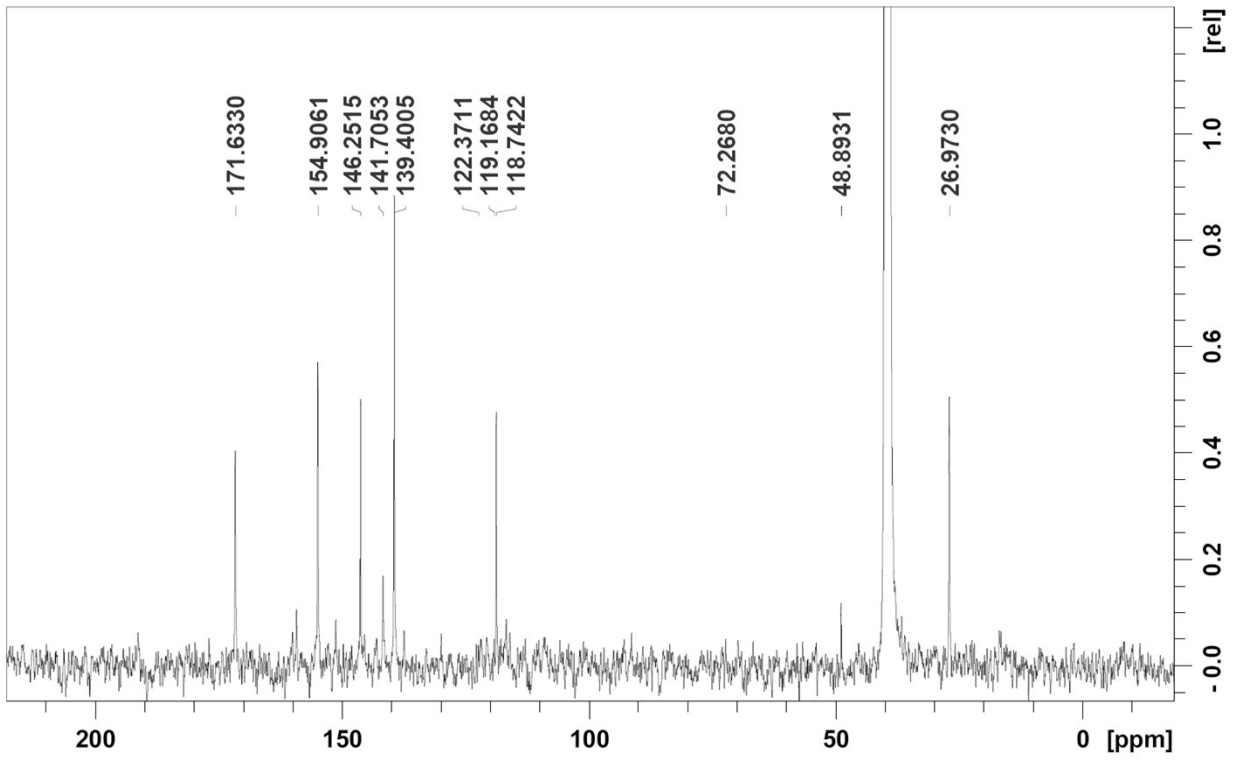


Bi(6-TG)$_3$·OTf

1. 400 MHz ^{1}H NMR in DMSO-d$_6$

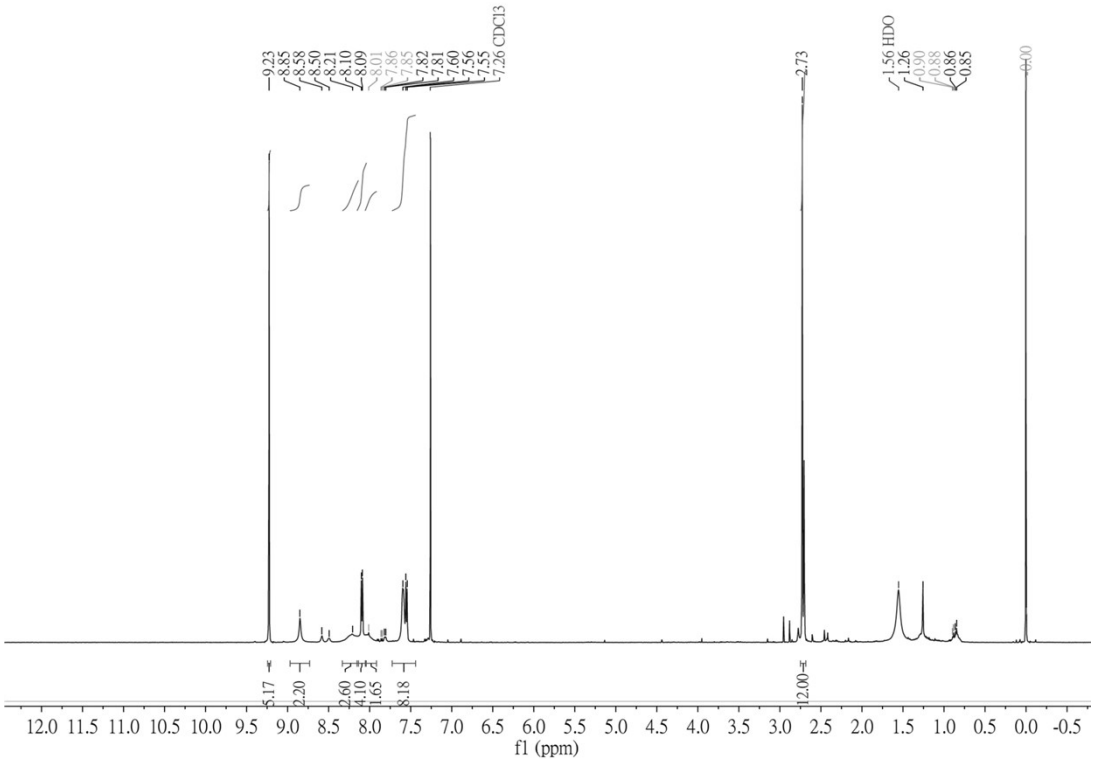


2. 100 MHz ^{13}C NMR in DMSO-d$_6$

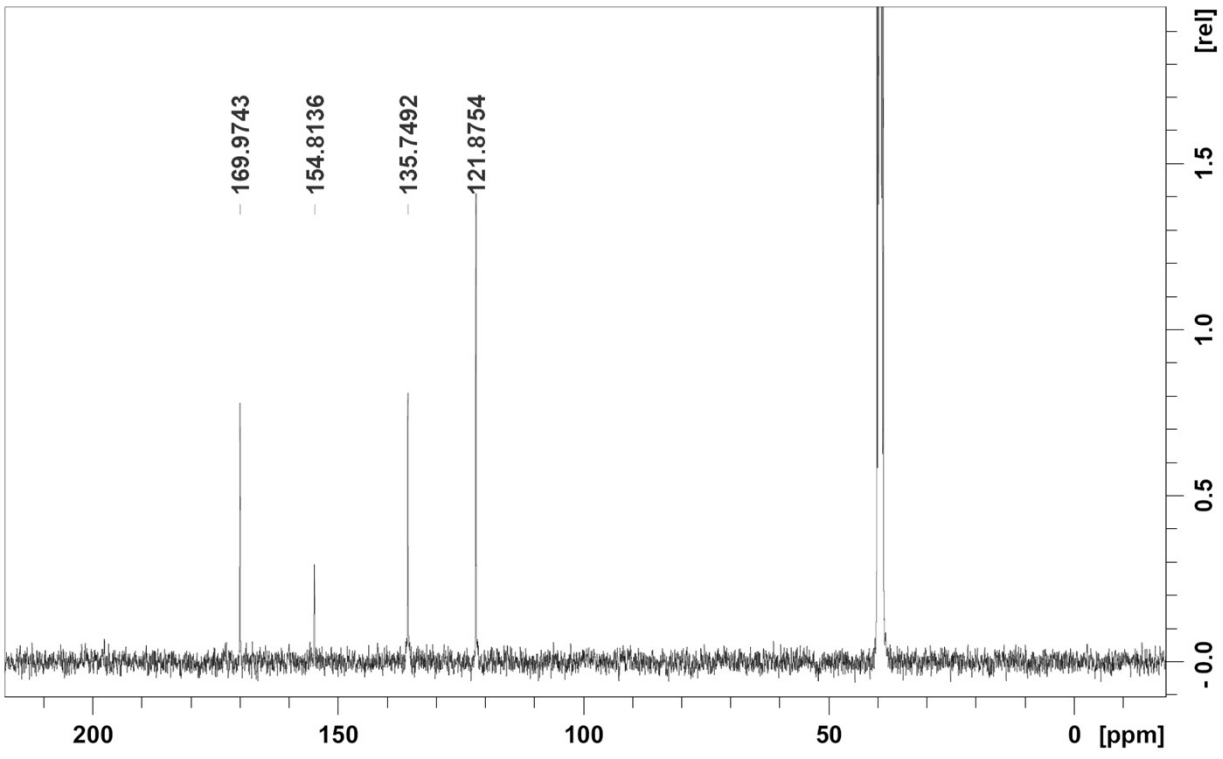


Bi(TMPP)

1. 500 MHz ^{1}H NMR in $CDCl_3$

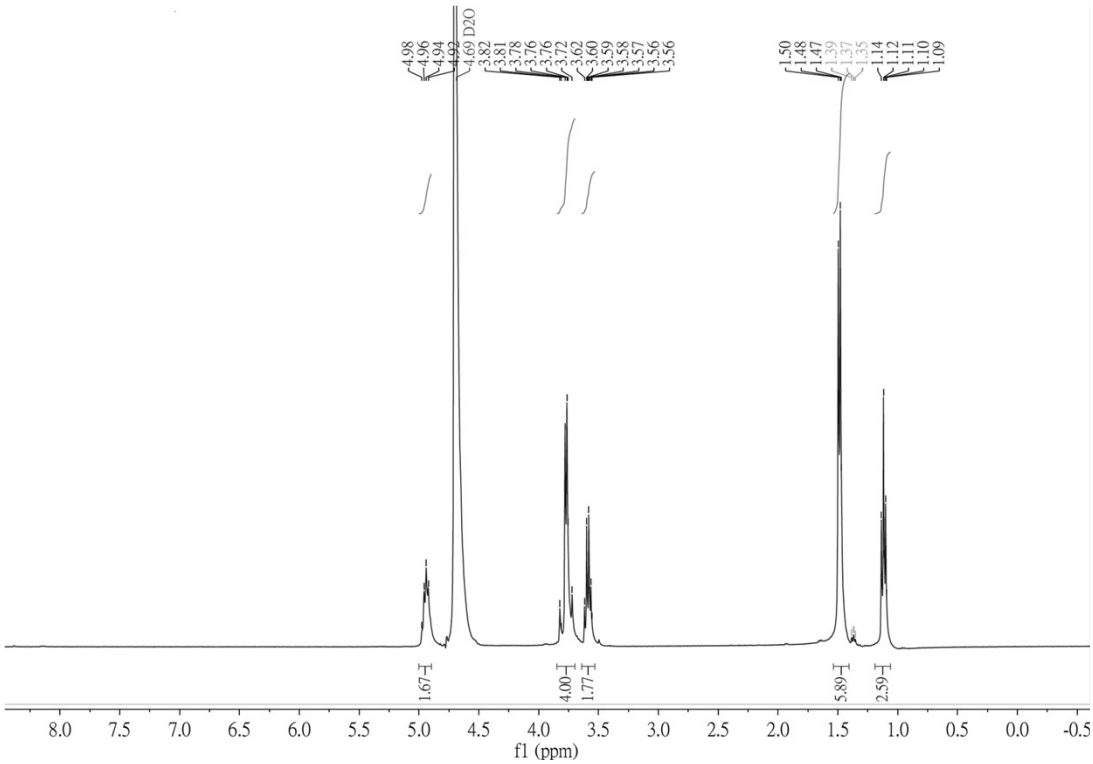


2. 100 MHz ^{13}C NMR in $CDCl_3$

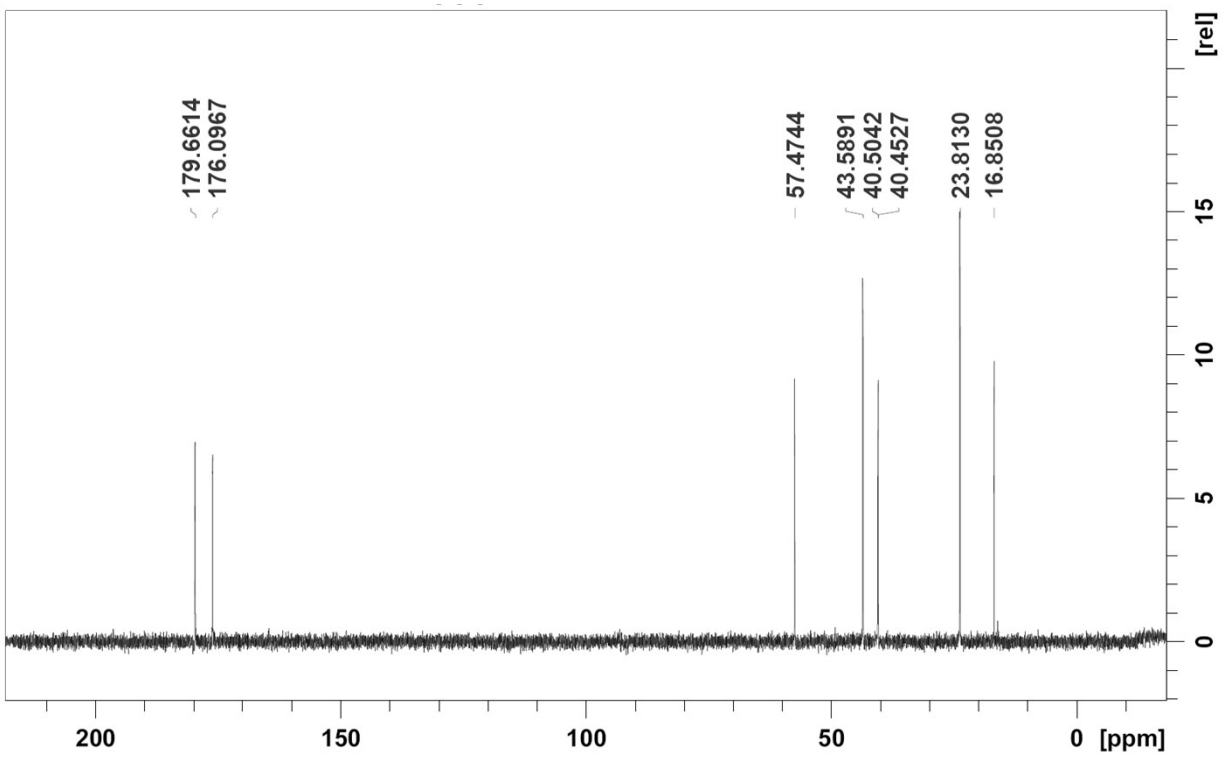


Bi(Tio)$_2$

1. 400 MHz ^{1}H NMR in D_2O

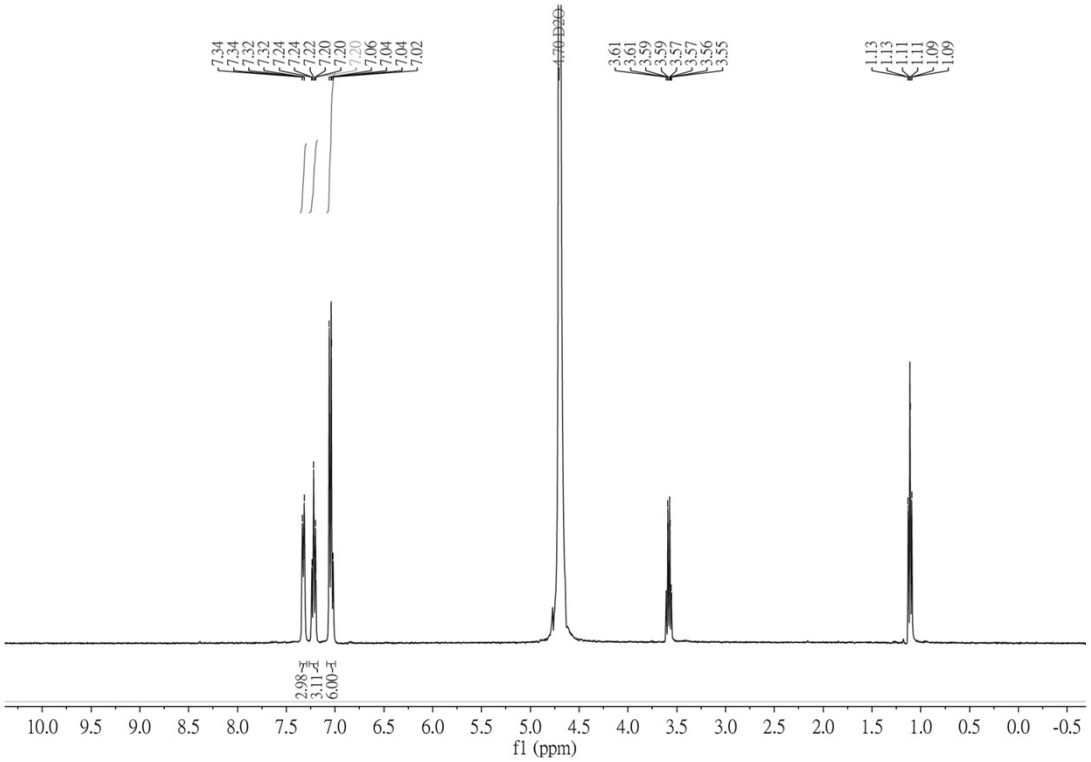


2. 400 MHz ^{13}C NMR in D_2O

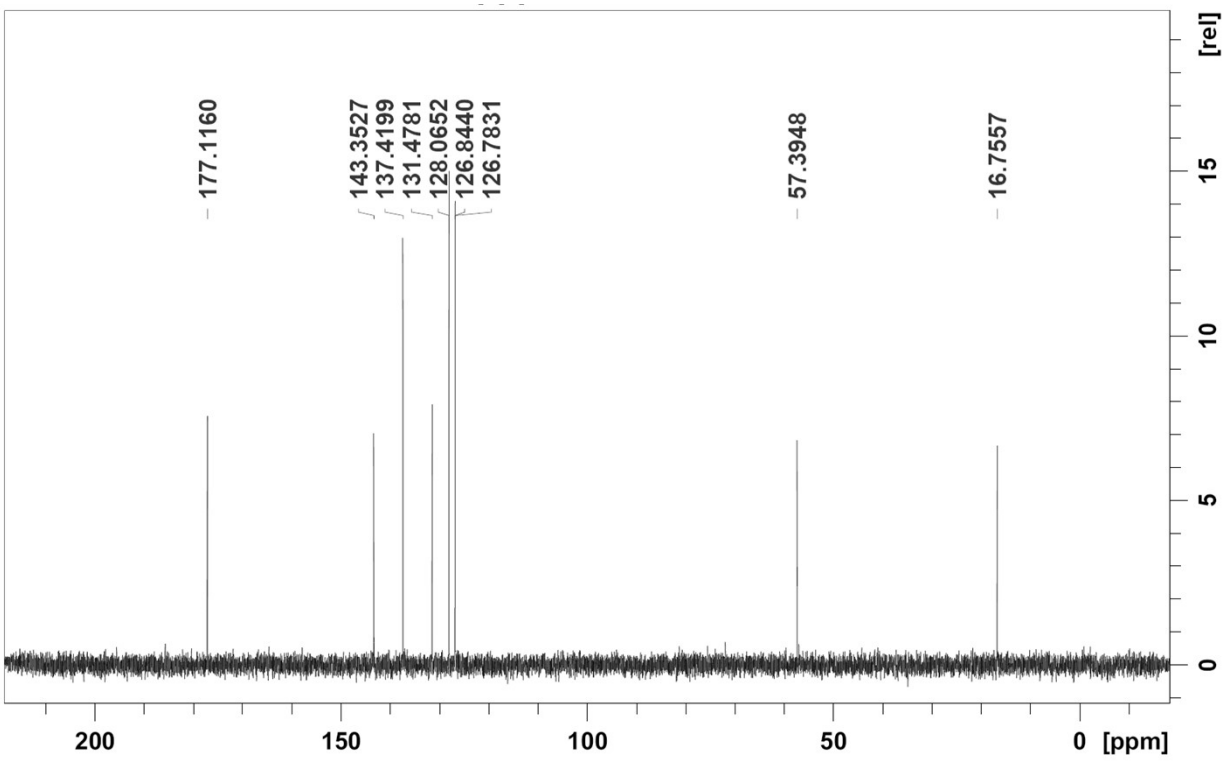


$Bi(TS)_3$

1. 400 MHz ^1H NMR in D_2O

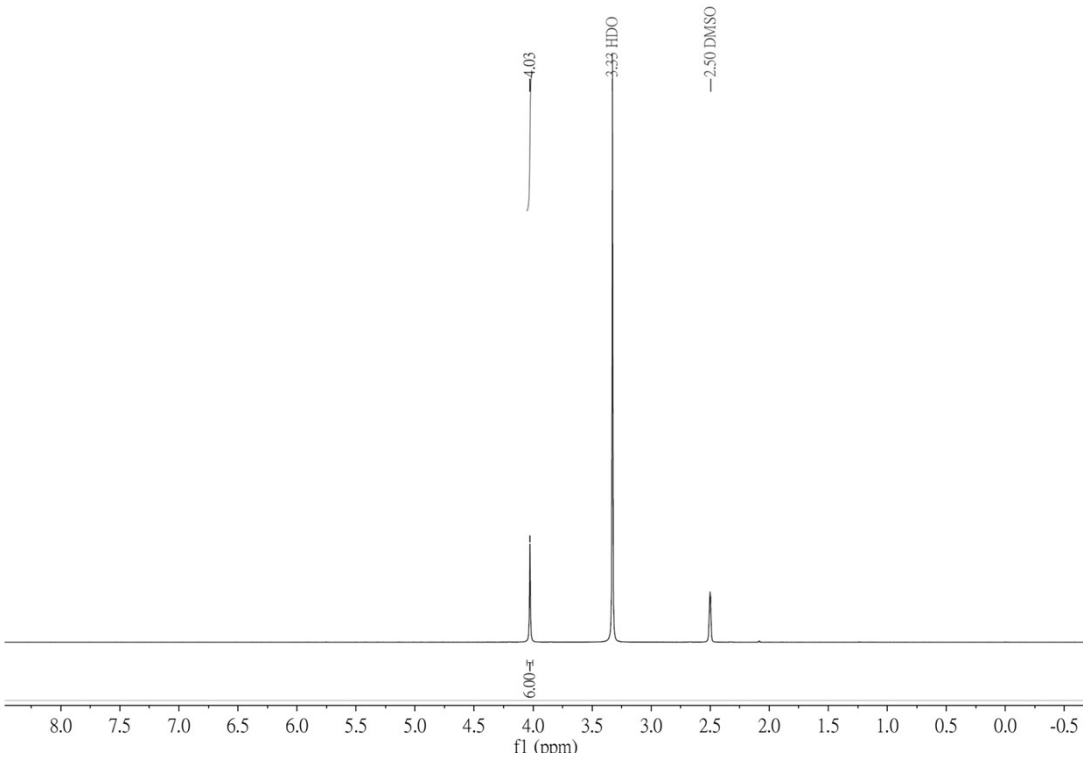


2. 100 MHz ^{13}C NMR in D_2O

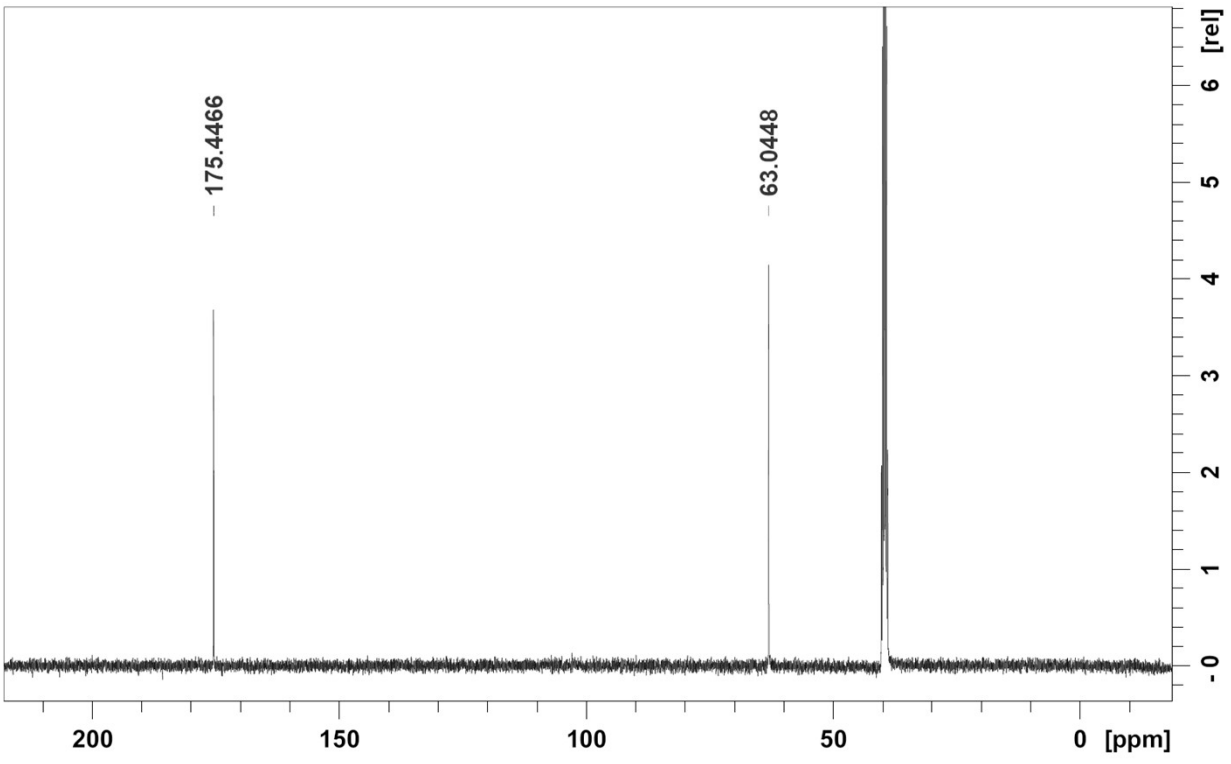


Bi(NTA)

1. 400 MHz ^{1}H NMR in DMSO-d_6

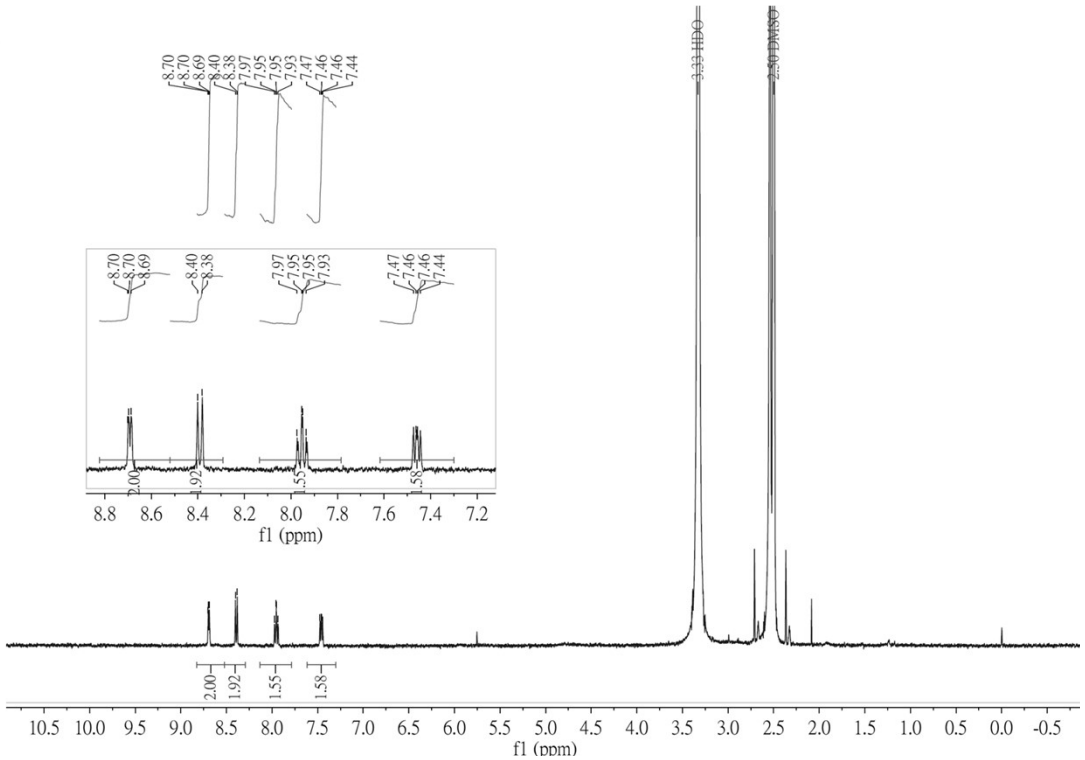


2. 100 MHz ^{13}C NMR in DMSO-d_6

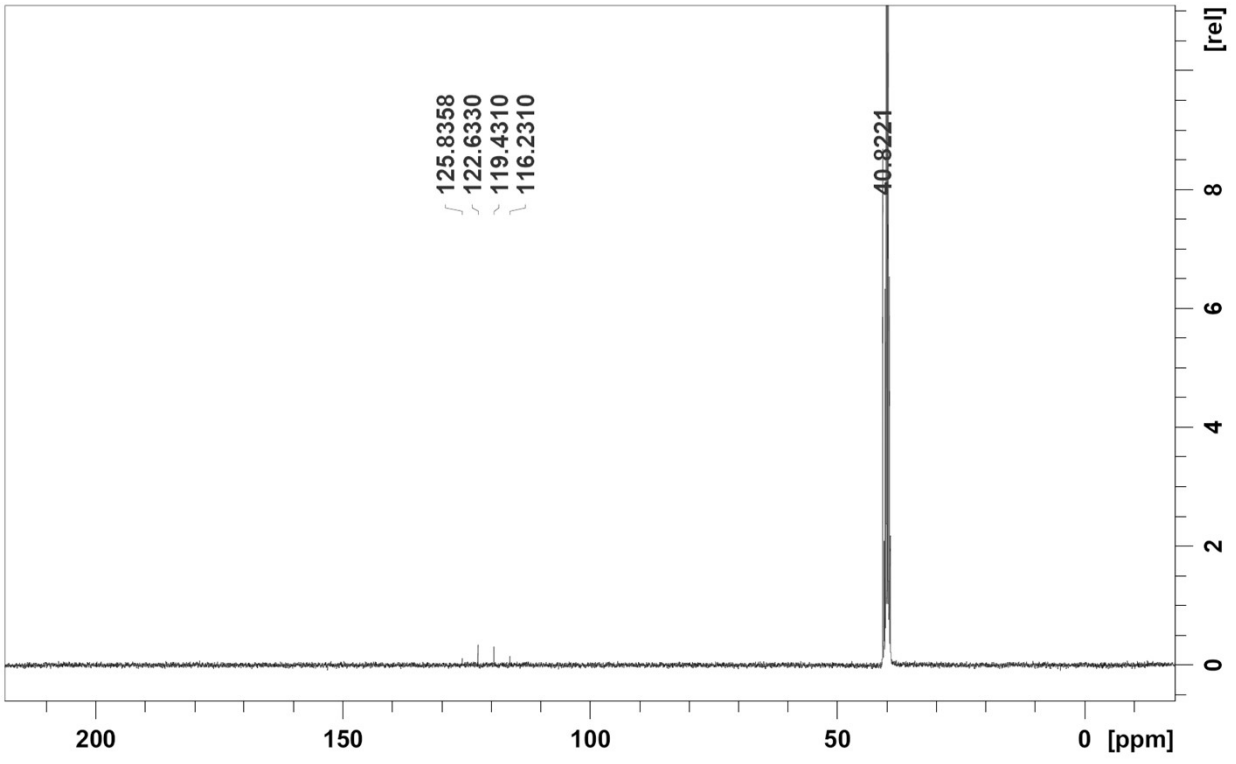


Bi(bpy)$_2$

1. 400 MHz ^{1}H NMR in DMSO-d$_6$

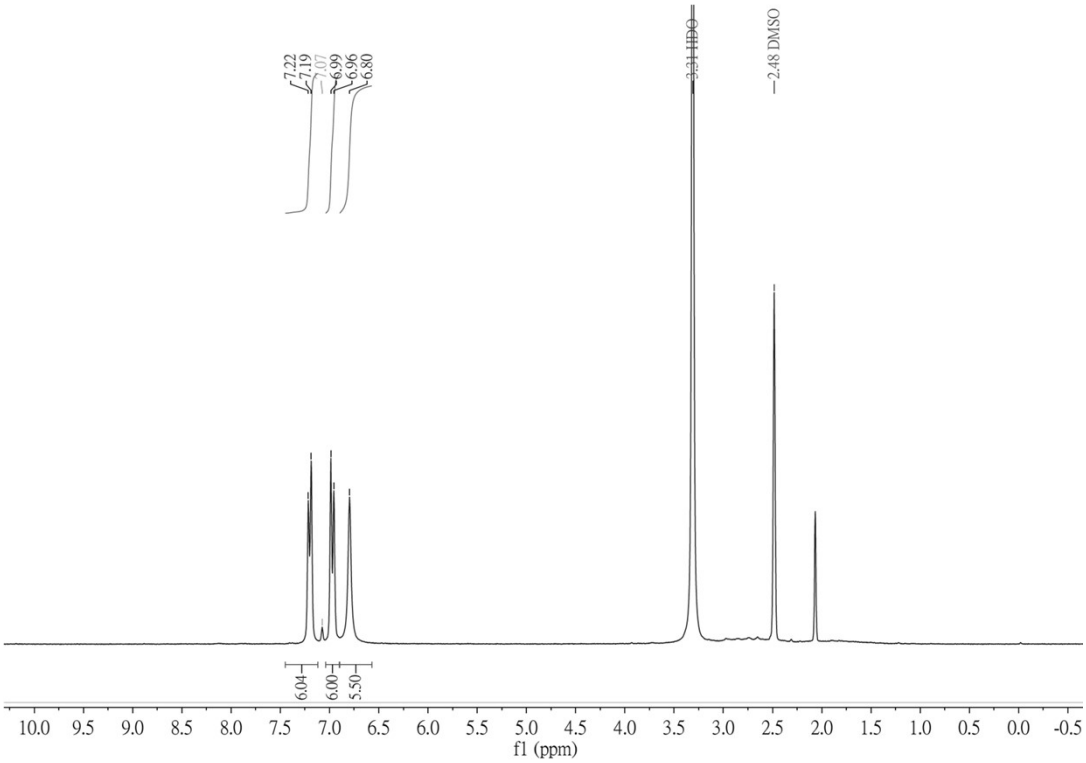


2. 100 MHz ^{13}C NMR in DMSO-d$_6$

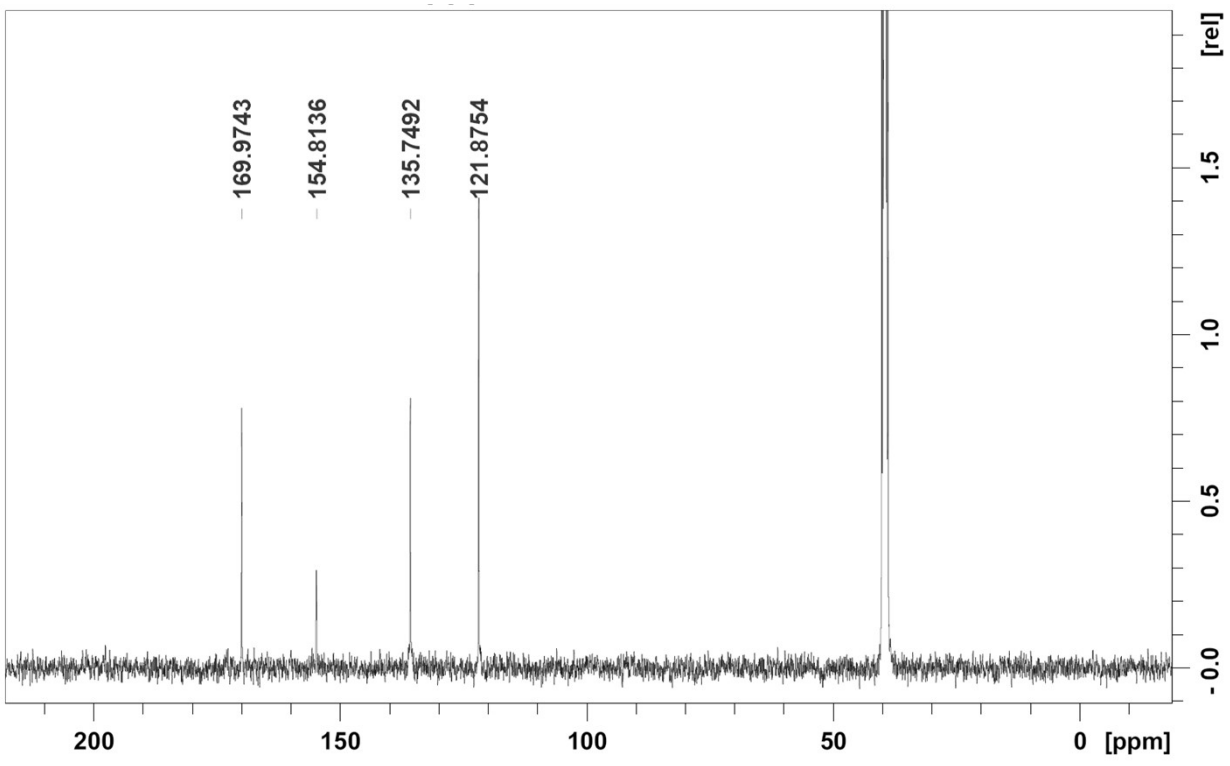


Bi(Tro-NH_2)$_3$

1. 400 MHz ^{1}H NMR in DMSO-d_6

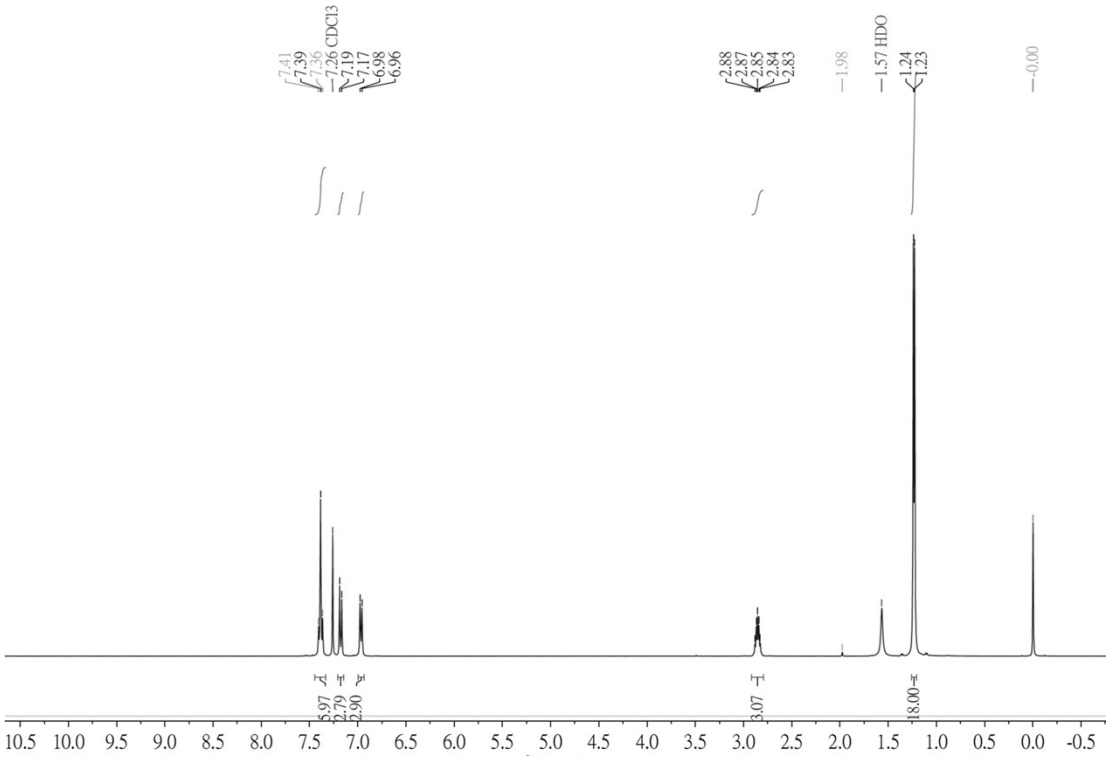


2. 100 MHz ^{13}C NMR in DMSO-d_6



Bi(Hino)$_3$

1. 500 MHz ^{1}H NMR in $CDCl_3$



2. 100 MHz ^{13}C NMR in $CDCl_3$

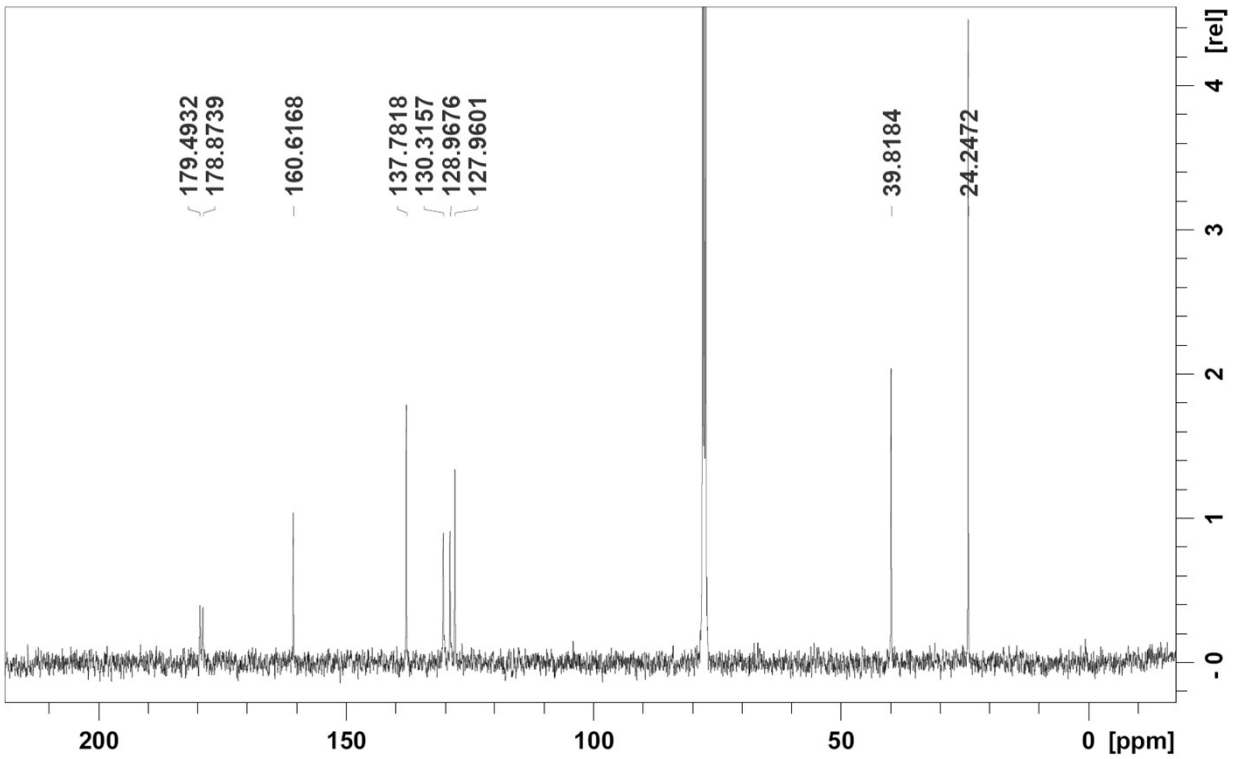
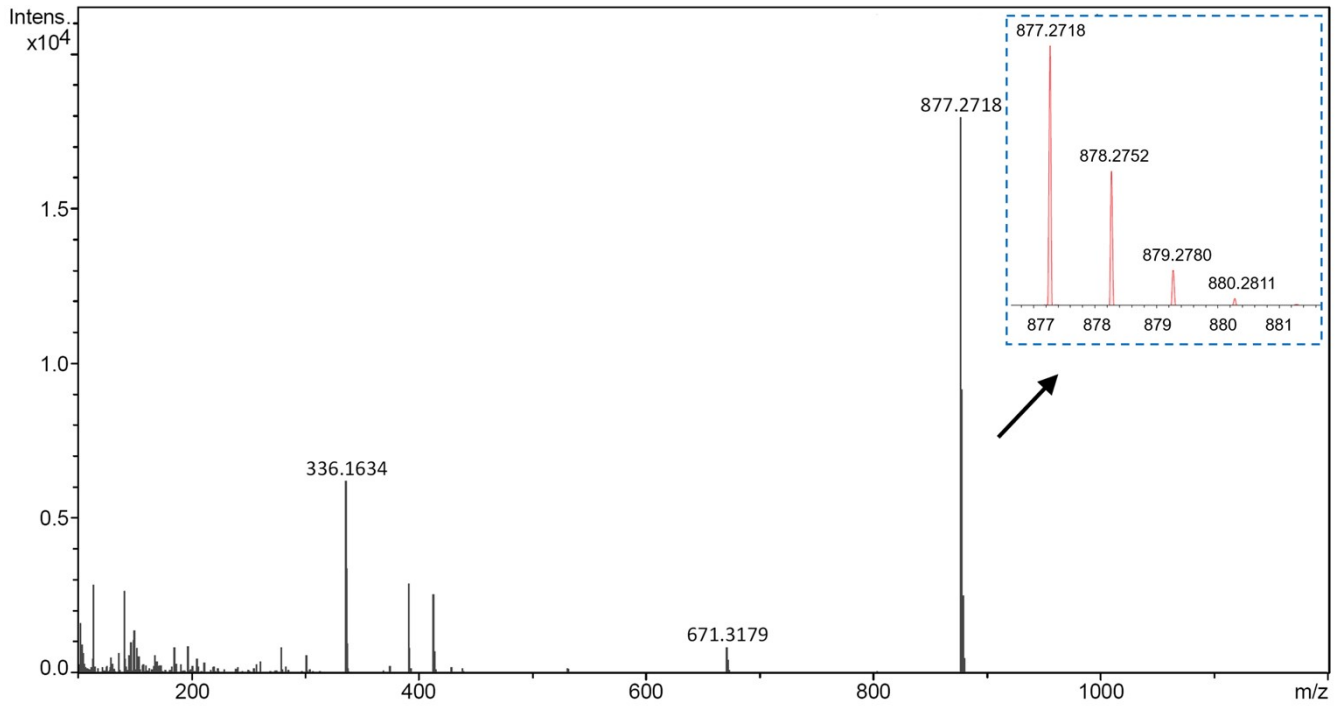


Figure S13. NMR spectra of the synthesized compounds.

HR-MS Spectra

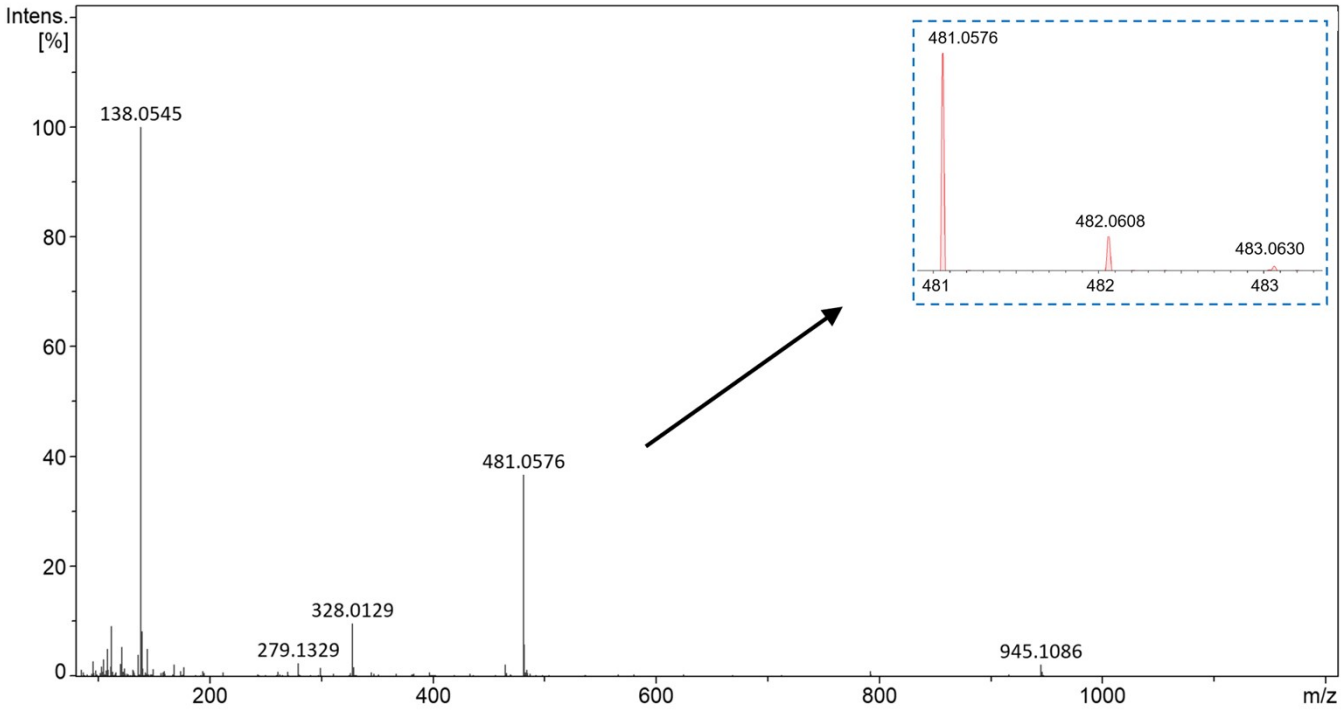
Bi(TMPP)

Solvent: 1% (v/v) chloroform diluted by ACN



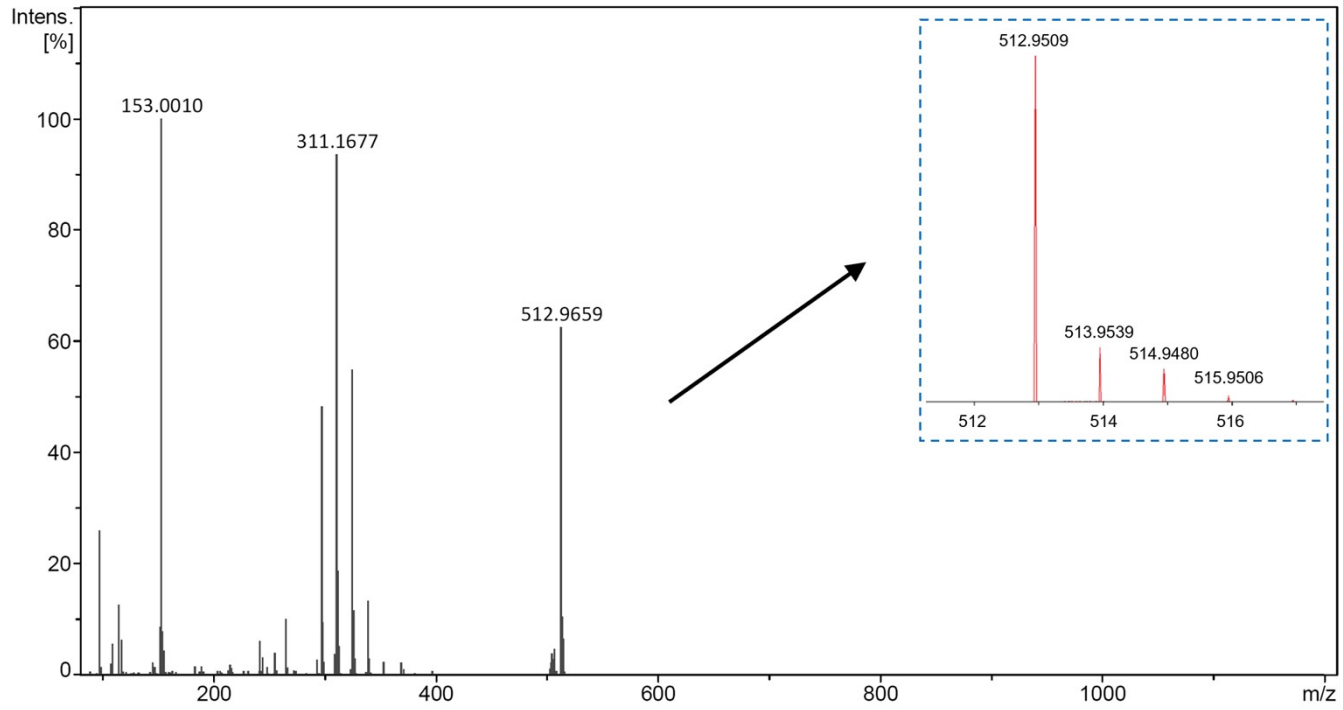
Bi(Tro-NH_2)$_3$

Solvent: MeOH



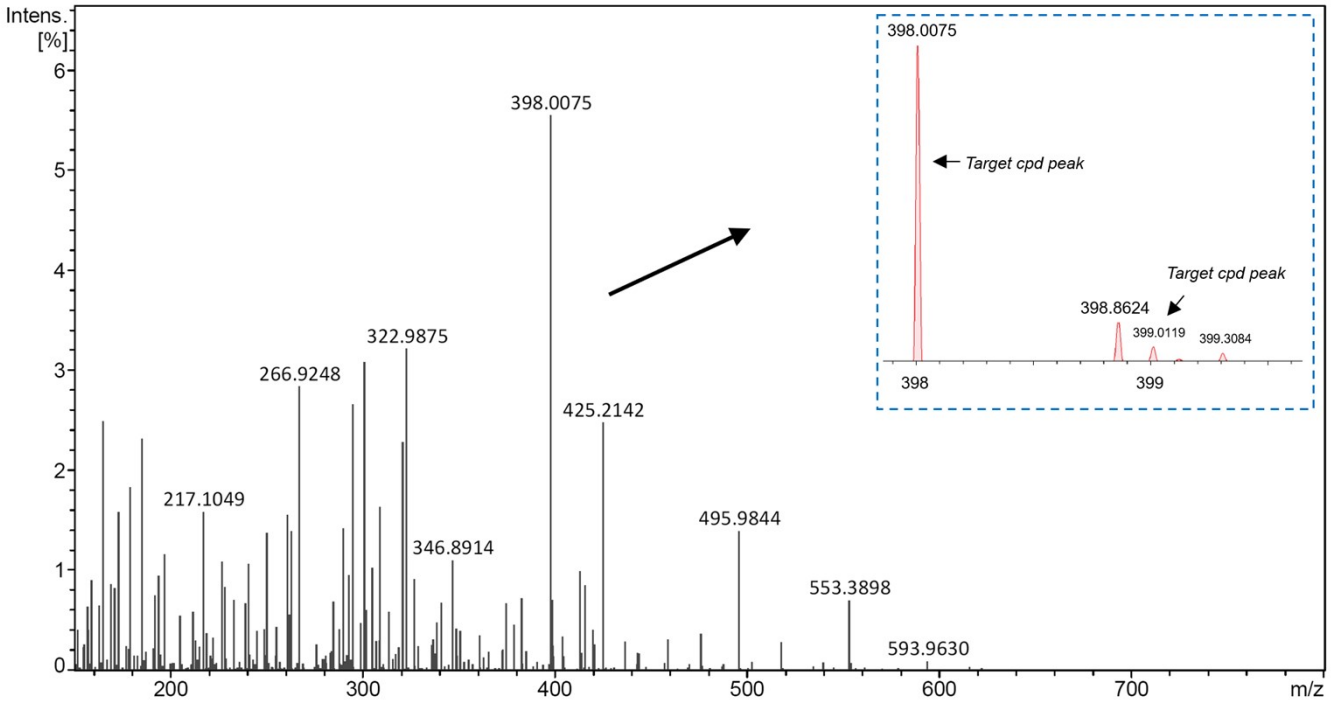
$Bi(TS)_3$

Solvent: Water



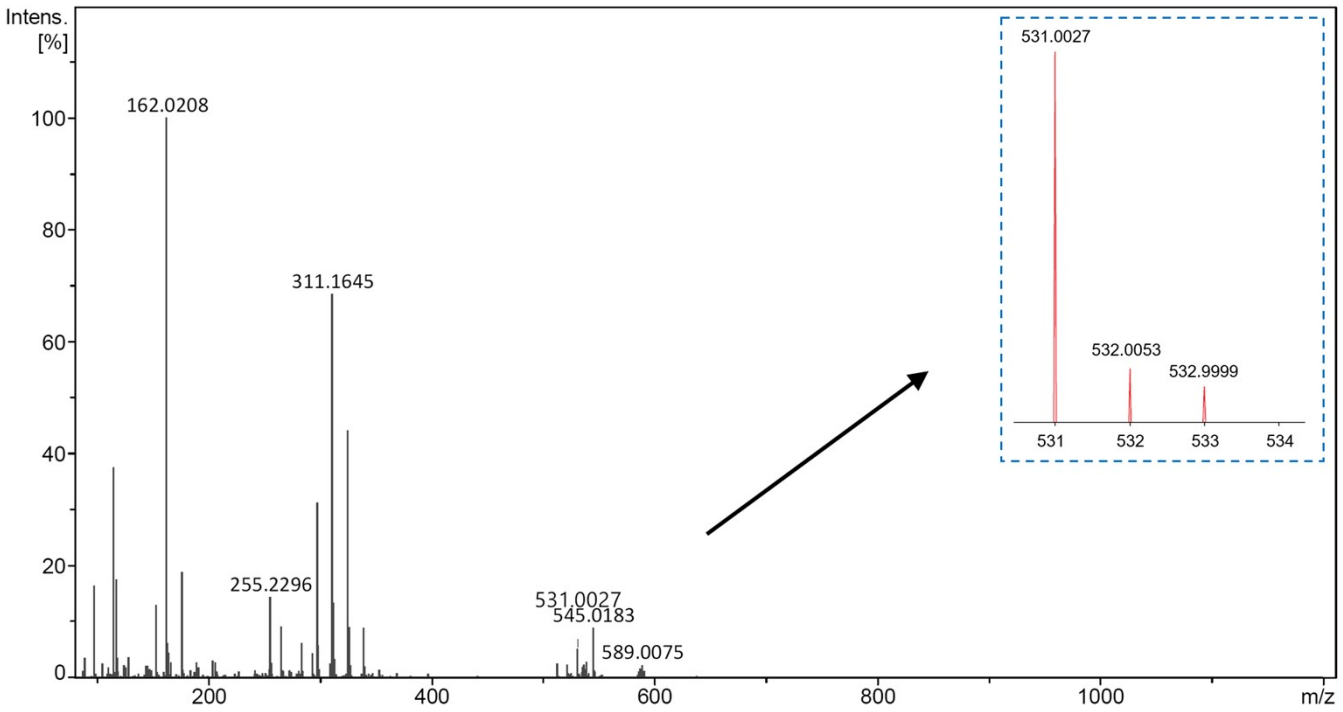
Bi(NTA)

Solvent: 1% (v/v) DMSO diluted by MeOH



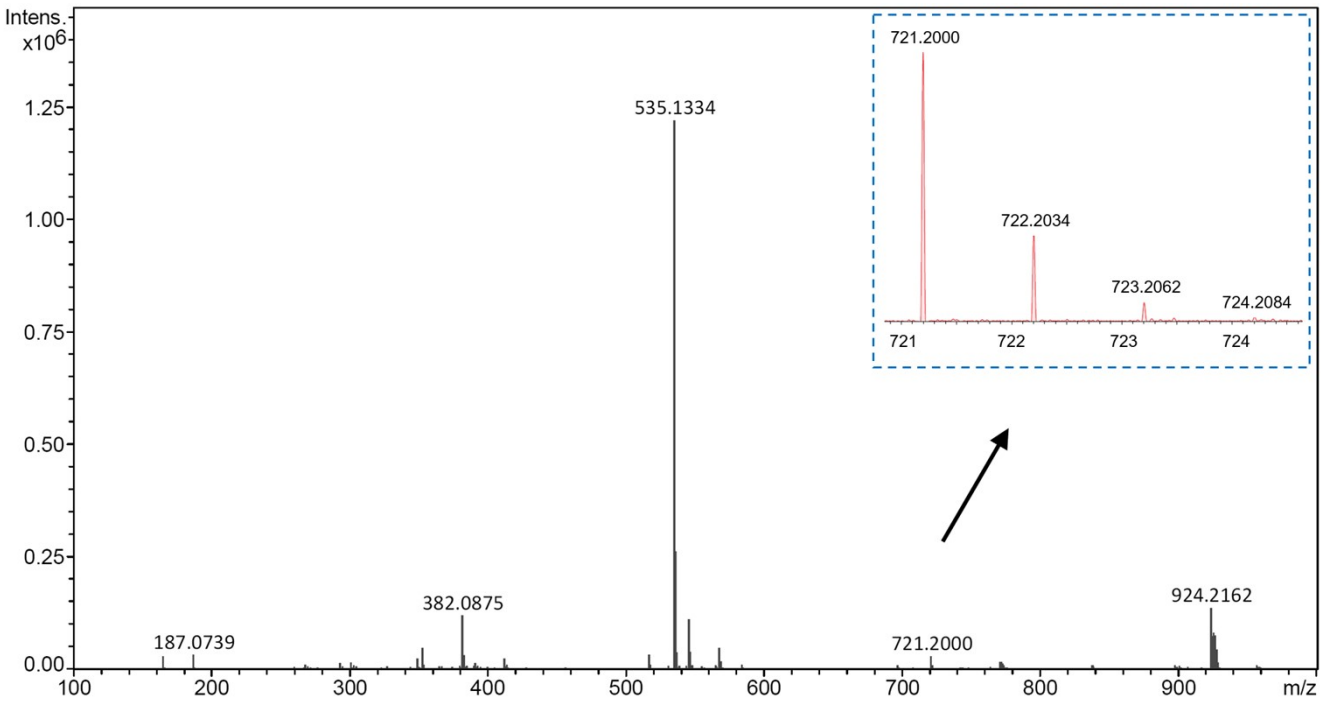
$Bi(Tio)_2$

Solvent: MeOH and water (1:1)



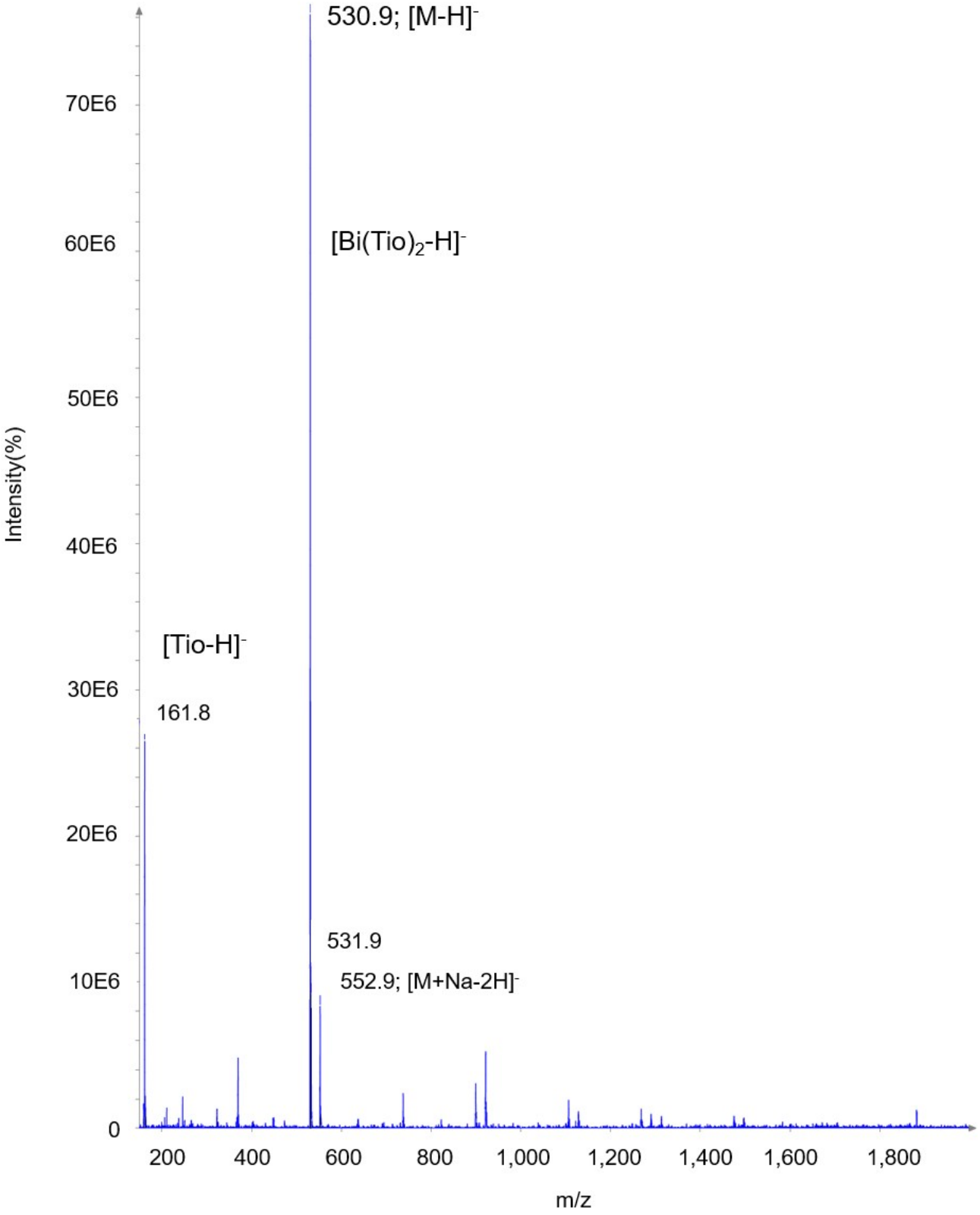
$Bi(Hino)_3$

Solvent: 1% (v/v) DMSO diluted by the mix of 1:1 MeOH and water



$Bi(Tio)_2$ LR-MS Spectra

Solvent: 1% MeOH (v/v) diluted by the mix of 9:1 acetonitrile (CAN) and water.



$Bi(Hino)_3$ LR-MS Spectra

Solvent: 1% DMSO (v/v) diluted by the mix of 9:1 ACN and water.

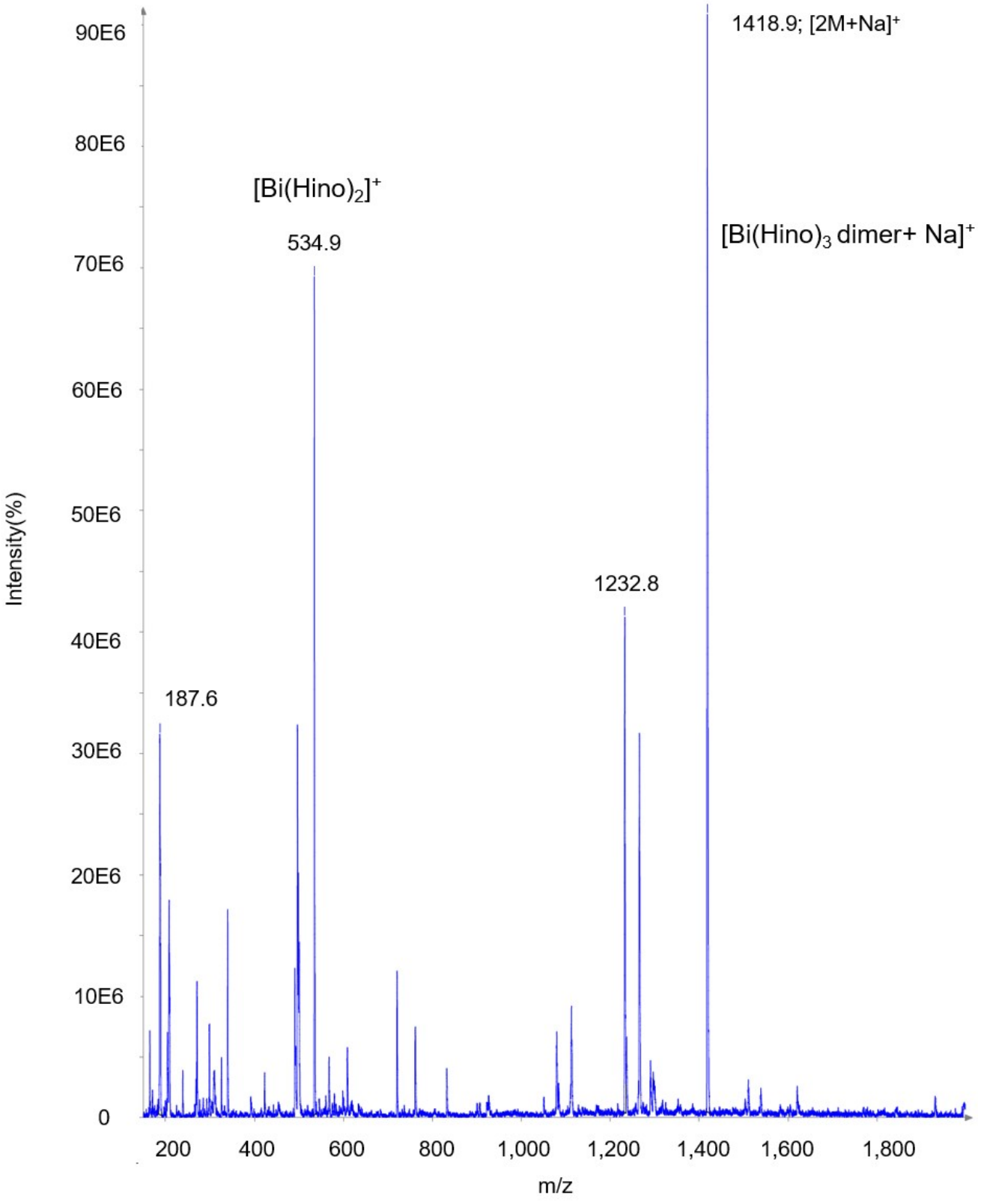
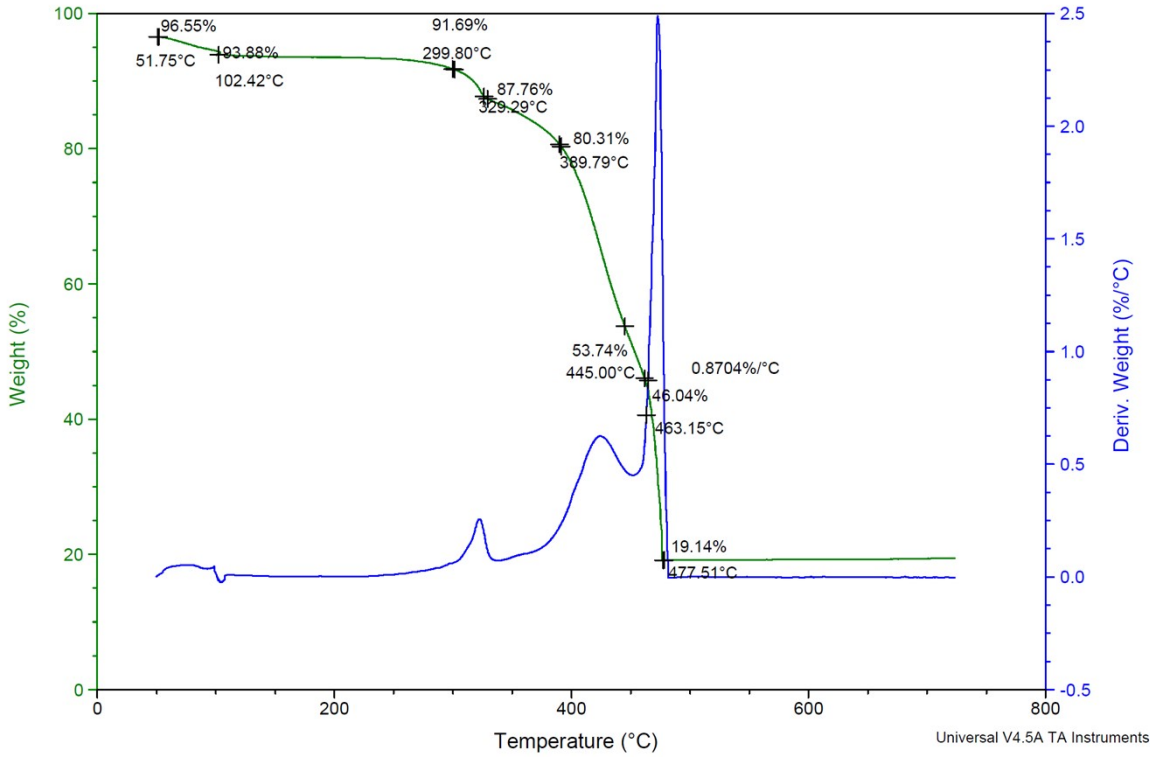


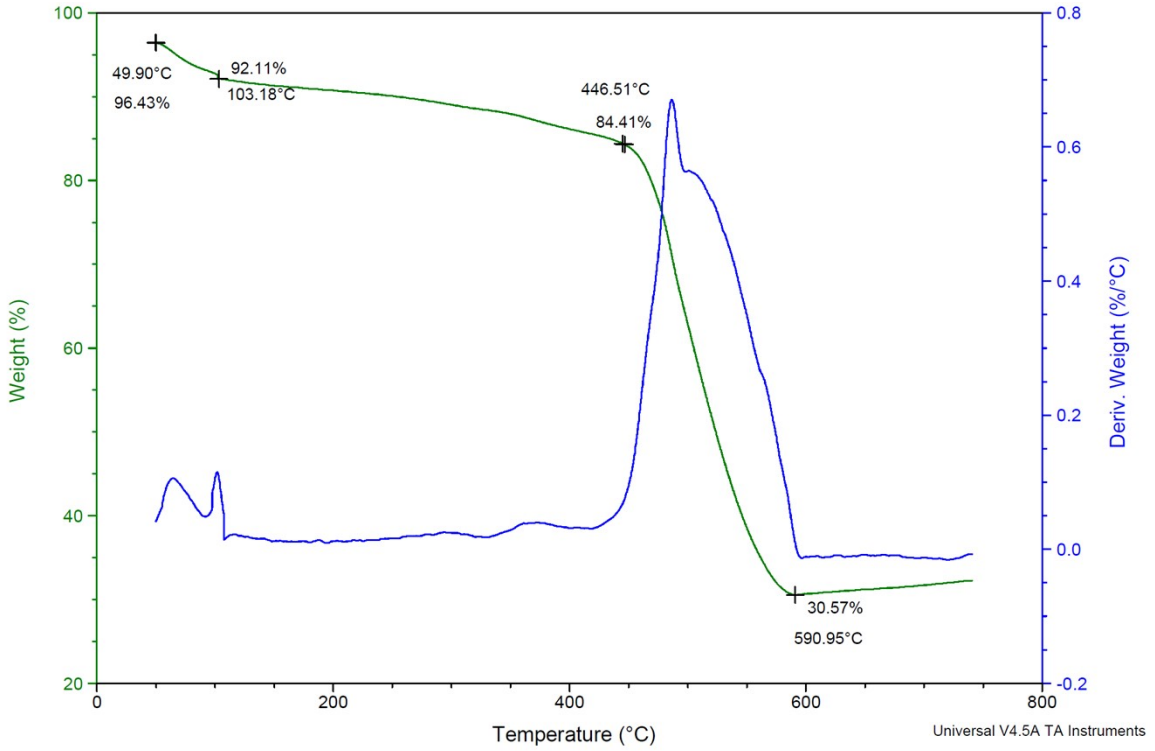
Figure S14. High resolution MS of selected novel synthesized compounds.

TG of metal compounds

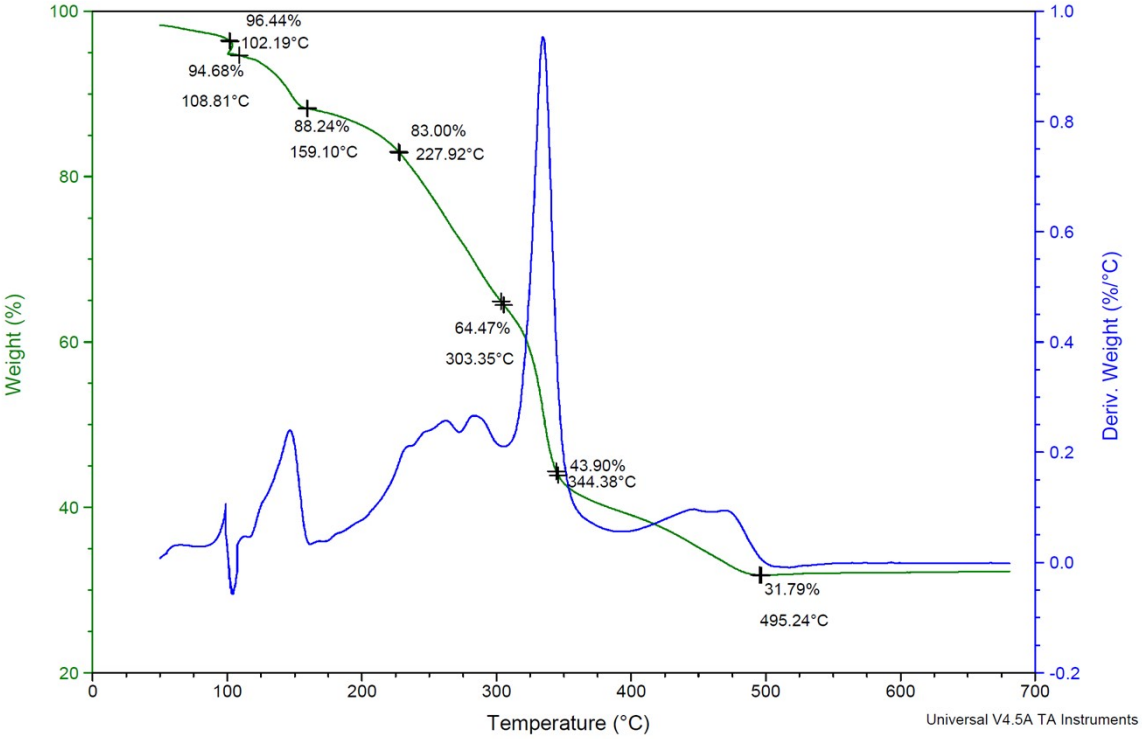
$Bi(6\text{-}TG)_3$



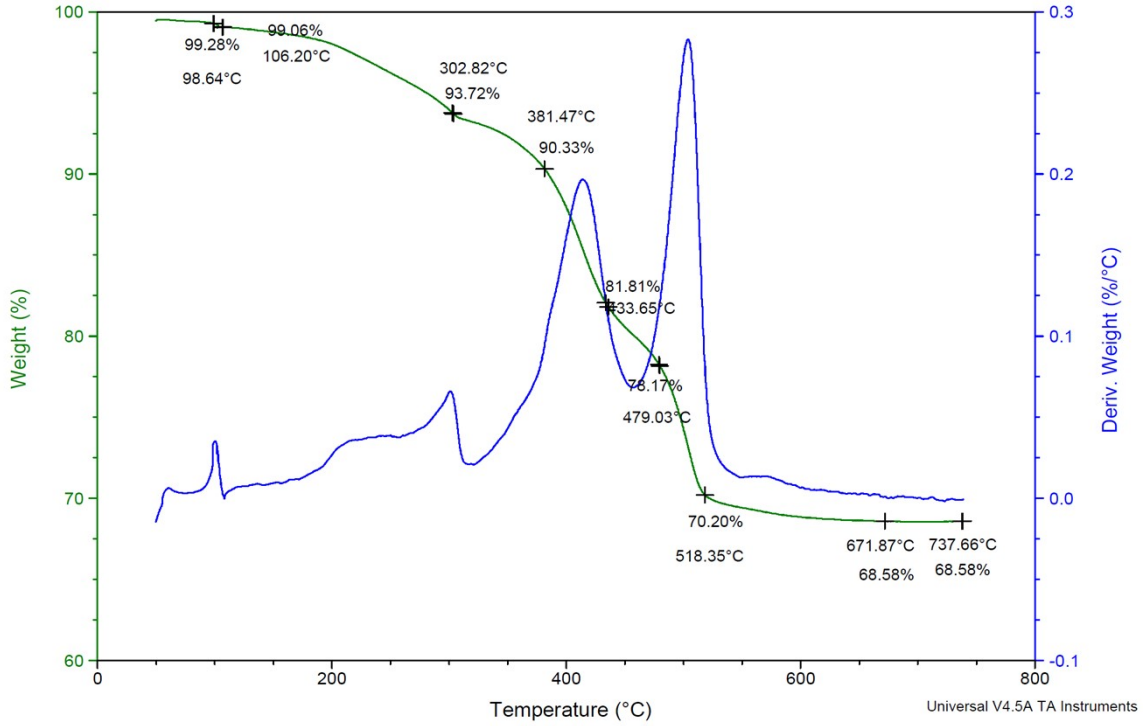
$Bi(6\text{-}TG)_3 \cdot OTf$



$Bi(TS)_3$



Bi(bpy)$_3$



Bi(Tro-NH$_2$)$_3$

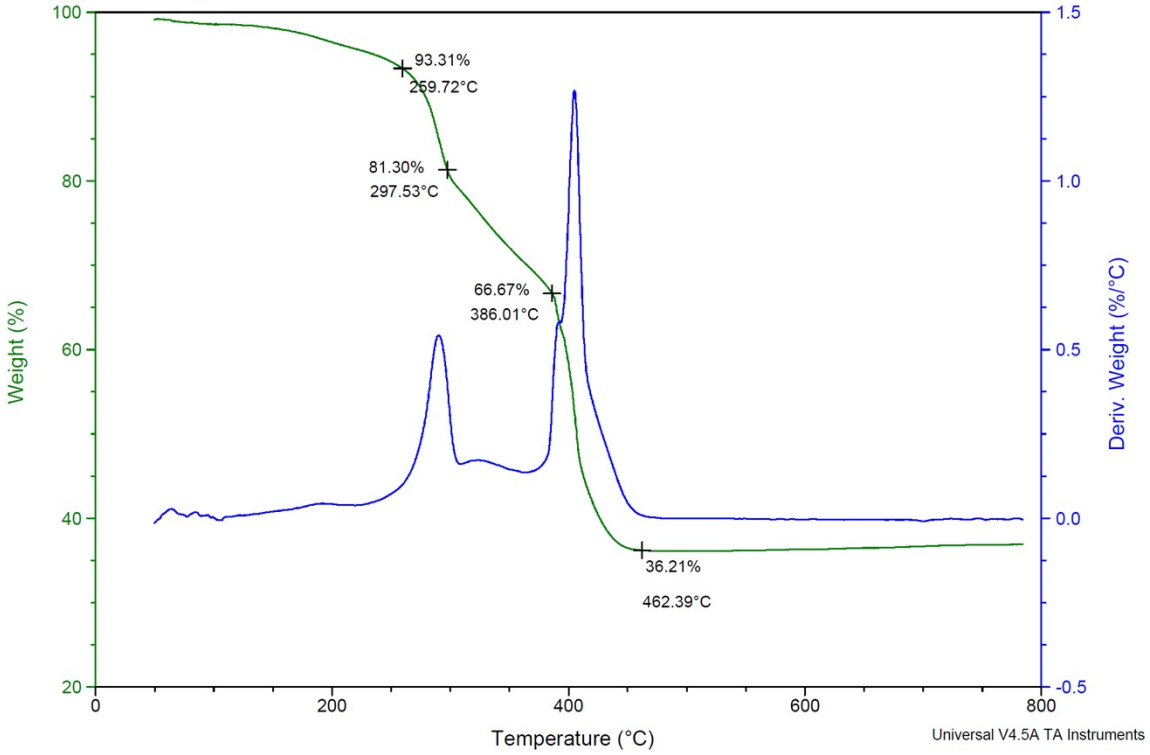


Figure S15. TG of selected novel synthesized compounds.

Table. S1 Calculated K_d of Bi(III) bound to different ligands

Bismuth-based Complex	LogK_a	K_d
$Bi(GSH)_3$	29.6±0.40*	$2.51(\pm2.31)x10^{-30}$
Bi(NTA)	17.55±0.34**	$2.82(\pm2.21)x10^{-18}$
$Bi(Tio)_2$	24.4±0.08	$3.98(\pm0.73)x10^{-25}$
$Bi(TS)_3$	33.5±0.54	$3.16(\pm3.92)x10^{-34}$
$Bi(Tro\text{-}NH_2)_3$	18.0±0.39	$1.00(\pm0.90)x10^{-18}$
$Bi(bpy)_2$	≤17.55	$\geq2.82x10^{-18}$

* This value is from P.J. Sadler et al *Chem Euro J*, 1996, **2**, 701. ** This value is from H. Li, et al, *J Biol Chem*, 1996, **271**, 9483.

Table. S2 Summary of selected bismuth compounds on their potency towards inhibition of ATPase and DNA unwinding activity of SARS-CoV-2 helicase as well as their cytotoxicity (CC_{50}) in VeroE6 cells.

Compounds	IC_{50} for ATPase activity (μM)	IC_{50} for unwinding activity (μM)	CC_{50} (VeroE6) (μM)
$Bi(GSH)_3$	2.99±1.78	0.64±0.09	>1000
Bi(TMPP)	3.39±0.32	1.22±0.69	268.30±1.99
$Bi(6\text{-}TG)_3$	0.85±0.15	2.96±0.51	>1000
$Bi(Tro\text{-}NH_2)_3$	0.03±0.01	0.36±0.02	186.50±16.55

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