Supporting Information

Hoechst-tagged radioiodinated BODIPY derivative for Auger-electron cancer therapy

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Materials and Methods

General remarks

Reagents purchased from commercial sources were used without further purification. The syntheses of compounds were carried under a nitrogen atmosphere. [^{125}I]NaI was purchased from Perkin Elmer (Boston, USA) as a non-carrier added solution in 0.1 M aqueous NaOH with specific activity of 629 GBq/mg. Purification of crude products was performed using Smart Flash EPCLC W-Prep 2XY (Yamazen Corporation, Osaka, Japan). Reversed phase high performance liquid chromatography (RP-HPLC) was carried on a Shimadzu system (an LC-20AT pump with an SPD-20A UV detector, λ = 254 nm; Shimadzu, Kyoto, Japan) with a Cosmosil C_{18} column ($5C_{18}$-AR-II, 4.6 × 150 or 10 × 250 mm; Nacalai Tesque, Kyoto, Japan). ^{1}H NMR spectra were obtained from a JEOL JNM-ECS400 system (JEOL, Tokyo, Japan) with tetramethylsilane as an internal standard. Coupling constants are reported in Hertz. Multiplicity was defined by s (singlet), d (doublet), t (triplet), q (quartet), br (broad), and m (multiplet). High-resolution mass spectra (HRMS) were conducted with LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Fluorescence parameters were obtained using UV-1800 (Shimadzu, Kyoto, Japan) and RF-6000 (Shimadzu, Kyoto, Japan).

Chemistry

Hoechst33258, I-BODIPY-NHS, and BODIPY-NHS were synthesized according to the previous procedure reported by Chandrika *et al.*[1], Ono *et al.*[2], and Nepomnyashchii *et al.*[3], respectively.

Compound 1

To a stirred suspension of Hoechst33258 (48.8 mg, 115 μmol) and K_2CO_3 (43.5 mg, 315 μmol) in *N,N*-dimethylformamide (DMF) (1 mL), *tert*-butyl (4-bromobutyl)carbamate (86.2 mg, 342 μmol) was added and the reaction mixture was heated at 60 ℃ and stirred overnight. The mixture was diluted with $CHCl_3$/washed with water and brine, and then dried over Na_2SO_4. The organic layer was evaporated under reduced pressure and the residue was purified by silica gel chromatography (MeOH:$CHCl_3$ = 1:4) to afford **1** (12.6 mg, 18.4%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.31-8.22 (m, 1H) 8.04 (d, *J* = 8.4 Hz, 2H) 7.97-7.89 (m, 1H) 7.61 (d, *J* = 8.0 Hz, 1H) 7.51 (d, *J* = 8.4 Hz, 1H) 7.21-7.12 (m, 1H) 7.07 (d, *J* = 9.2 Hz, 2H) 7.04-7.00 (m, 1H) 4.08-4.05 (t, *J* = 6.0 Hz, 2H) 3.30-3.26 (m, 4H) 3.14-3.10 (t, *J* = 7.2 Hz, 2H) 2.86-2.71 (m, 4H) 2.46 (s, 3H) 1.84-1.80 (m, 2H) 1.68-1.64 (m, 2H) 1.44 (s, 9H). HRMS (ESI) *m/z* calcd for $C_{34}H_{42}N_7O_3^+$, 596.3344 $[M+H]^+$: found 596.3340.

Compound 2 (BH)

Trifluoroacetic acid (1 mL) was added to a solution of **1** (12.6 mg, 21.2 μmol) in CH_2Cl_2 (1.5 mL). The reaction mixture was stirred at room temperature for 1 h. The mixture was evaporated under reduced pressure. The residue was dissolved with DMF (1.5 mL), and then I-BODIPY-NHS (12.5 mg, 21.1 μmol) and *N*,*N*-diisopropylethylamine (DIPEA) (31 μL, 178 μmol) were added to the solution. The reaction mixture was stirred at room temperature for 7 h. The mixture was diluted with $CHCl_3$/and washed with water and brine, and then dried over Na_2SO_4. The organic layer was evaporated under reduced pressure and the residue was purified by silica gel chromatography (MeOH:$CHCl_3$ = 1:4) to afford **2** (4.3 mg, 20.9%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.36-8.28 (m, 1H) 8.09 (d, *J* = 8.4 Hz, 2H) 8.01-7.96 (m, 1H) 7.89 (d, *J* = 8.0 Hz, 2H) 7.82 (d, *J* = 8.4 Hz, 1H) 7.71 (d, *J* = 9.2 Hz, 1H) 7.40 (d, *J* = 7.6 Hz, 2H) 7.37-7.34 (m, 1H) 7.32-7.29 (m, 1H) 7.18 (d, *J* = 9.2 Hz, 2H) 6.11 (s, 1H) 4.26-4.20 (m, 2H) 3.55-3.49 (m, 2H) 3.29-3.28 (m, 4H) 3.24-3.18 (m, 4H) 3.02 (s, 3H) 2.48 (s, 3H) 2.45 (s, 3H) 1.98-1.87 (m, 4H) 1.36 (s, 3H) 1.33 (s, 3H). HRMS (ESI) *m/z* calcd for $C_{49}H_{50}BF_2IN_9O_2^+$, 972.3118 $[M+H]^+$: found 972.3110.

Compound 3 (BH precursor)

Trifluoroacetic acid (1.5 mL) was added to a solution of **1** (26.6 mg, 44.7 μmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at room temperature for 1 h. The mixture was evaporated under reduced pressure. The residue was dissolved with DMF (2 mL), and then

BODIPY-NHS (20 mg, 43 μmol) and DIPEA (63 μL, 362 μmol) were added to the solution. The reaction mixture was stirred at 50 ℃ for 6 h. The mixture was diluted with $CHCl_3$/washed with water and brine, and then dried over Na_2SO_4. The organic layer was evaporated under reduced pressure and the residue was purified by silica gel chromatography (MeOH:$CHCl_3$ = 1:4) to afford **3** (7.6 mg, 20.9%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.30-8.25 (m, 1H) 8.06 (d, *J* = 8.8 Hz, 2H) 7.99-7.96 (m, 1H) 7.93 (d, *J* = 8.0 Hz, 2H) 7.72-7.65 (m, 1H) 7.52 (d, *J* = 8.8 Hz, 1H) 7.42 (d, *J* = 8.0 Hz, 2H) 7.19-7.15 (m, 1H) 7.12 (d, *J* = 8.8 Hz, 2H) 7.07 (d, *J* = 8.0 Hz, 1H) 5.99 (s, 2H) 4.22-4.14 (m, 2H) 3.57-3.47 (m, 2H) 3.29-3.23 (m, 4H) 2.84-2.73 (m, 4H) 2.45 (s, 3H) 2.44 (s, 6H) 1.98-1.84 (m, 4H) 1.35 (s, 6H). HRMS (ESI) *m/z* calcd for $C_{49}H_{51}BF_2N_9O_2^+$, 846.4221 $[M+H]^+$: found 846.4229.

Compound 4 (BD)

To a solution of I-BODIPY-NHS (38.7 mg, 65.4 μmol) in CH_2Cl_2 (10 mL), 4-aminobutanol (6.1 μL, 65.7 μmol) and DIPEA (93.5 μL, 537 μmol) were added dropwise and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with $CHCl_3$/washed with water and brine, and then dried over Na_2SO_4. The organic layer was evaporated under reduced pressure and the residue was purified by silica gel chromatography (AcOEt:Hexane = 5:1) to afford **4** (33 mg, 89.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.0

Hz, 2H) 7.30 (d, *J* = 8.0 Hz, 2H) 6.65 (br s, 1H) 5.98 (s, 1H) 3.70 (t, *J* = 6.0 Hz, 2H) 3.49 (q, *J* = 6.4 Hz, 2H) 2.57 (s, 3H) 2.50 (s, 3H) 1.88-1.62 (m, 4H) 1.30 (s, 3H) 1.29 (s, 3H). HRMS (ESI) *m/z* calcd for $C_{24}H_{28}BF_2IN_3O_2^+$, 566.1282 $[M+H]^+$: found 566.1280.

Compound 5 (BD precursor)

To a solution of BODIPY-NHS (115.4 mg, 248 μmol) in CH_2Cl_2 (10 mL), 4-aminobutanol (23 μL, 248 μmol) and DIPEA (353 μL, 2027 μmol) were added dropwise and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with $CHCl_3$/washed with water and brine, and then dried over Na_2SO_4. The organic layer was evaporated under reduced pressure and the residue was purified by silica gel chromatography (AcOEt:Hexane = 5:1) to afford **5** (89.6 mg, 82.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.0 Hz, 2H) 7.32 (d, *J* = 8.4 Hz, 2H) 6.54 (br s, 1H) 5.92 (s, 2H) 3.70 (t, *J* = 5.6 Hz, 2H) 3.49 (q, *J* = 6.4 Hz, 2H) 2.49 (s, 6H) 1.77-1.62 (m, 4H) 1.29 (s, 6H). HRMS (ESI) *m/z* calcd for $C_{24}H_{29}BF_2N_3O_2^+$, 440.2315 $[M+H]^+$: found 440.2317.

General procedure for the radioiodination

N-Chlorosuccinimide in MeOH (50 μL, 0.5 mg/mL) was added to a mixture of a corresponding precursor in MeOH containing 1% CH_3COOH (25 μL, 1 mg/mL) and 6.85 MBq

of [^{125}I]NaI. The reaction mixture was vortexed and allowed to react for 30 min at room temperature, and quenched by the addition of 50 μL of H_2O and 100 μL of saturated $NaHSO_3$, followed by neutralization with saturated $NaHCO_3$. The reaction mixture was extracted with ethyl acetate (400 μL×3). The combined organic layers were evaporated under a stream of nitrogen. The residues were purified by RP-HPLC. The collected fractions containing products were evaporated to dryness under a stream of nitrogen. The identification of radioiodinated products was performed by coelution with reference compounds using a Cosmosil 5C_{18}-AR-II column (4.6 × 150 mm) eluted at a flow rate of 1.0 mL/min (Figure S1).

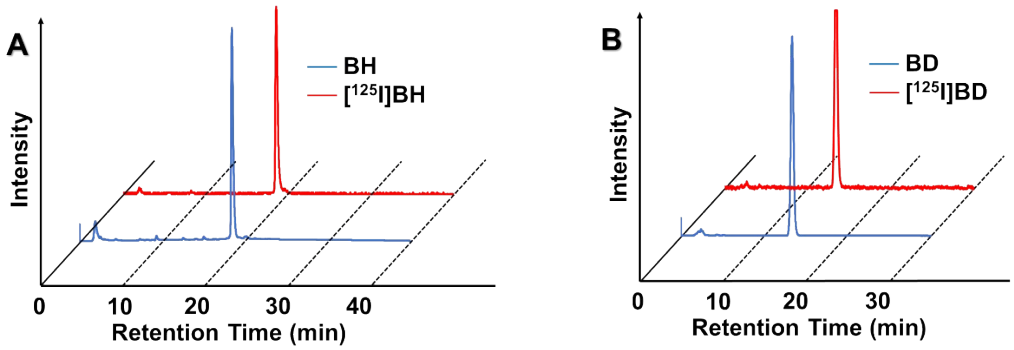


Figure S1. RP-HPLC coelution of [^{125}I]BH with nonradioactive BH (**A**) and [^{125}I]BD with BD (**B**). Mobile phase condition: (**A**) H_2O / MeCN / Trifluoroacetic acid = 70 / 30 / 0.1 → 20 / 80 / 0.1 (0-40 min, gradient). (**B**) H_2O / MeCN / Trifluoroacetic acid = 50 / 50 / 0.1.

Determination of fluorescence parameters

Absorption and emission spectra were recoded using UV-1800 and RF-6000.

Fluorescence quantum yields (Φ_f) were measured and calculated using the following equation:

$\Phi_f = (F_s / F_{ref)} \cdot (n_s^2 / n_{ref}^2) \cdot (A_{ref} / A_s) \cdot \Phi_{ref}$

wherein F, A/and n represent the area under emission peak of fluorescence, absorbance at the excitation position (525 nm), and refractive index of the solvent, respectively. I-BODIPY-NHS[2] was used as a reference in toluene ($\Phi_f = 0.11$) (Table S1).

Table S1. Fluorescence parameters of BH and BD

Compound	λ_{abs} (nm)[a]	λ_{em} (nm)[a]	ε (M^{-1} cm^{-1})[a]	Φ_f[a]
BH	517	532	22000	0.06
BD	517	532	39800	0.07

[a]In MeOH

Cell culture

HeLa, Human cervical cancer cells (JCRB, Japan), were cultured in Gibco Minimum Essential Media (EMEM) containing 1% non-essential amino acid supplemented with 10% fetal bovine serum albumin and 1% penicillin-streptomycin antibiotic solution in a humidified atmosphere of 95% air and 5% CO_2 at 37 ℃.

Nuclear uptake

HeLa cells, 1.0×10^6 per well/were seeded in a 6-well plate and allowed to attach

overnight. The cells were incubated at 37°C for a period of 2, 4, or 6 h with 18.5 kBq of [^{125}I]BH or [^{125}I]BD in 2 mL of assay media (Dulbecco's Modified Eagle Medium (DMEM) with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.5% MeOH and 0.2% bovine serum albumin (BSA)). After incubation, cells were removed from the plates by scraping and collected with radioactive media into a 2-mL tube. The radiocompounds in the media were removed by centrifugation of the cell suspension at 2100 *g* for 3 min at 4 °C, followed by washing the cellular pellet with ice-cold phosphate buffered saline (PBS). The radioactivity of the cellular pellet was measured using a γ-counter (Wizard2470 PerkinElmer) to quantify the total cellular uptake of the radiocompounds. The cellular pellet was suspended by 2 mL of ice-cold cell lysis buffer (10 mM Tris, 1.5 mM $MgCl_2$, 140 mM NaCl) containing 0.1% of IGEPAL-ca 630 and incubated on ice for 10 min to remove the cell membrane. After incubation, the cell suspension was centrifuged at 1300 *g* for 2 min at 4 °C, the supernatant was separated from the pellet (nuclei) and the radioactivity in the pellet was measured with a γ-counter. The results were evaluated by Student's *t*-test.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

HeLa cells, 1.0×10^4 per well/were added to a 96-well plate and allowed to attach overnight. Cells were incubated with 0-37 kBq of [^{125}I]BH, [^{125}I]BD, 0-4570 pM of BH/or BD for

2 h at 37 ℃. Subsequently, MTT reagents were added to each well, which was then incubated for 4 h at 37 ℃. After incubation, MTT-containing medium was removed/ the cells were washed with PBS, and then isopropyl alcohol containing 0.04 M HCl aq was added to dissolve MTT formazan crystals. Absorbance at 570 nm was measured using a microplate reader (iMark™, Bio-Rad, California Hercules, USA). The results were evaluated by two-way ANOVA with Sidak's multiple comparison post-test using Graph Pad Prism version 6 software (GraphPad Software, California San Diego, USA).

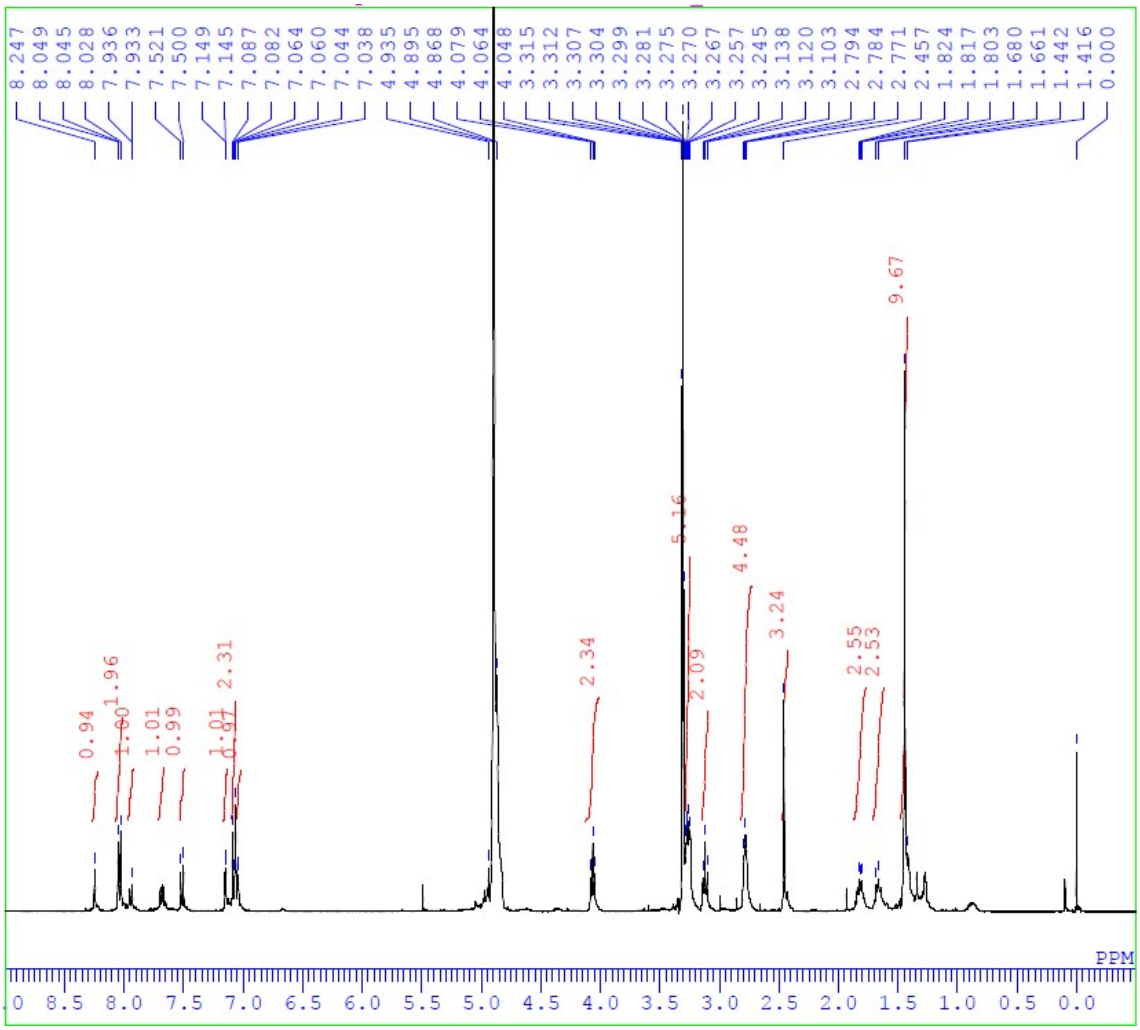
γ-H2AX assay

HeLa cells, 1.0×10^4 per well/were added to a 16-well chamber slide and allowed to attach overnight. Cells were incubated with radiocompounds for 2 h at 37 ℃. After incubation, cells were washed twice with PBS and fixed with 4% formaldehyde for 5 min. After washing, cells were permeabilized with 1% Triton X-100 in PBS for 20 min. After further washing, cells were incubated with anti-γ-H2AX primary antibody solution (Dojindo, Kumamoto, Japan) for 1 h. After subsequent washing, cells were incubated with FITC-conjugated secondary antibody solution (Dojindo, Kumamoto, Japan) for 1 h. Then, after the last washing, cells were finally incubated with DAPI at 1 μg/mL for 15 min. Images of about 200 nuclei per treatment were taken with florescence microscopy, BZ-9000 (KEYENCE, Osaka, Japan). γ-H2AX foci were analyzed

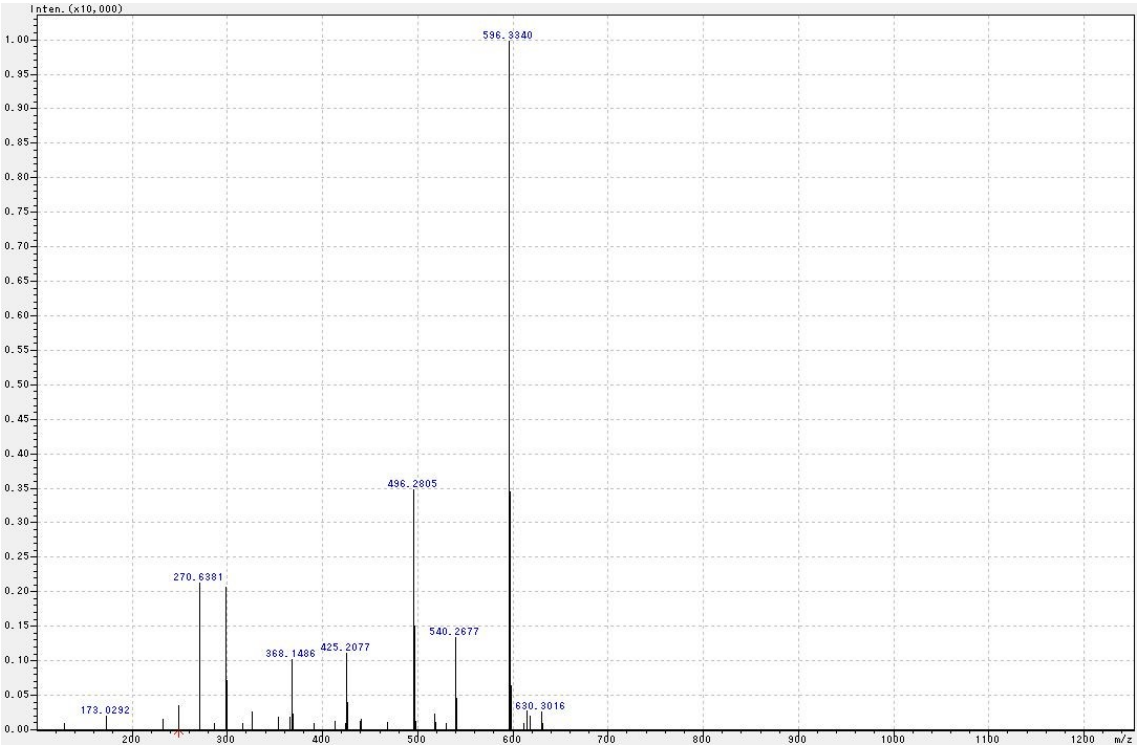
by BZ-II analysis application, and quantified by measuring the integrated density of foci per unit area of the nucleus (sum of mean grey density of focus × focus area divided by nucleus area). The results were evaluated by two-way ANOVA with Tukey's multiple comparison using GraphPad Prism 6 software.

NMR and HRMS spectra

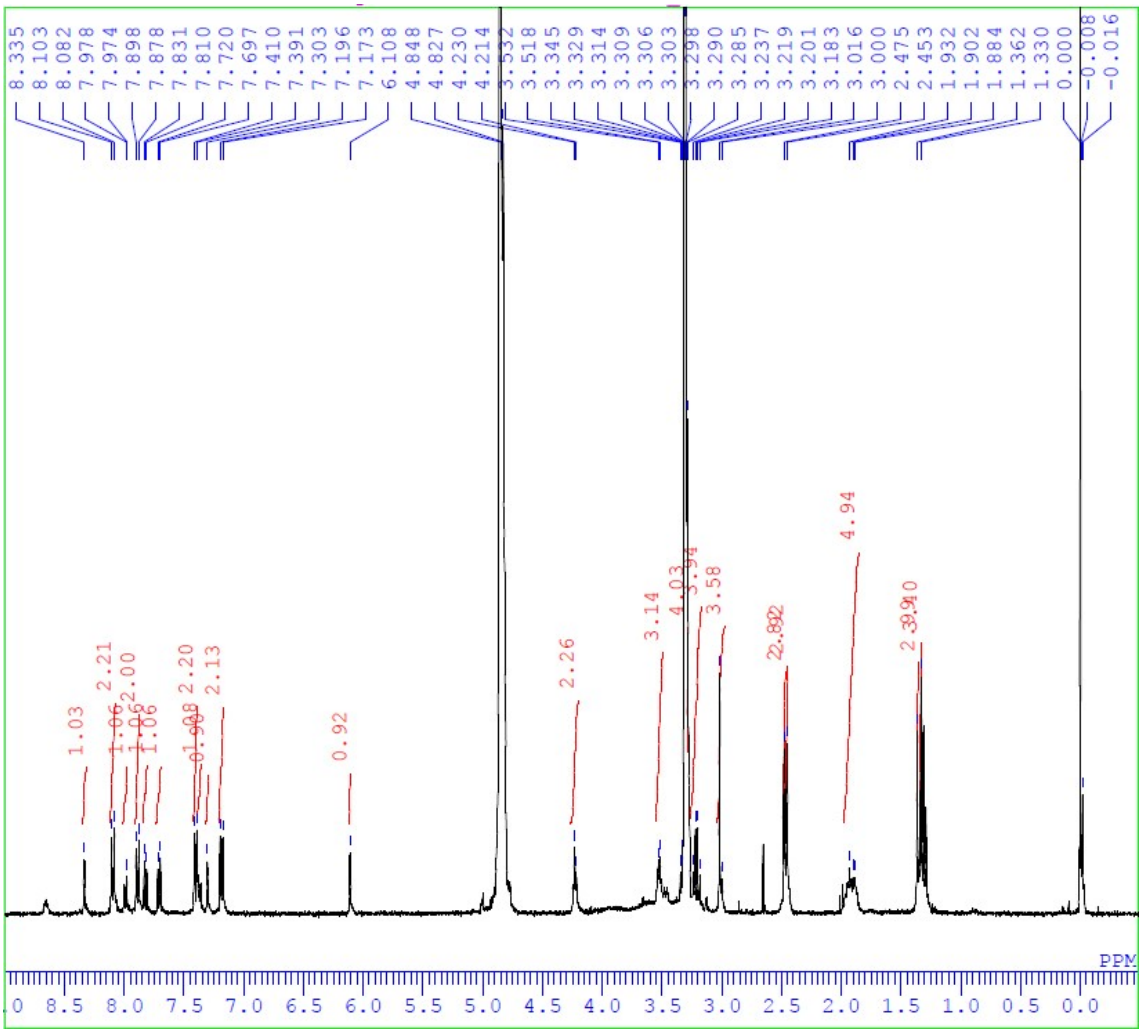
^{1}H NMR spectrum for **Compound 1**



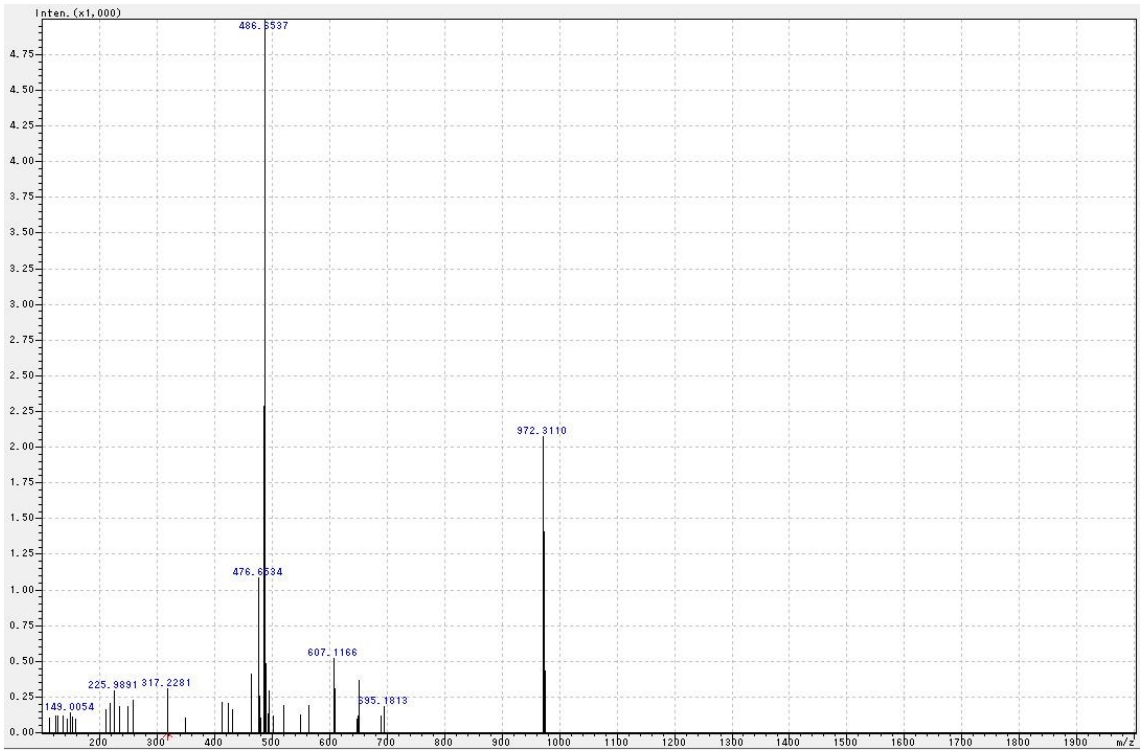
HRMS spectrum for **Compound 1**



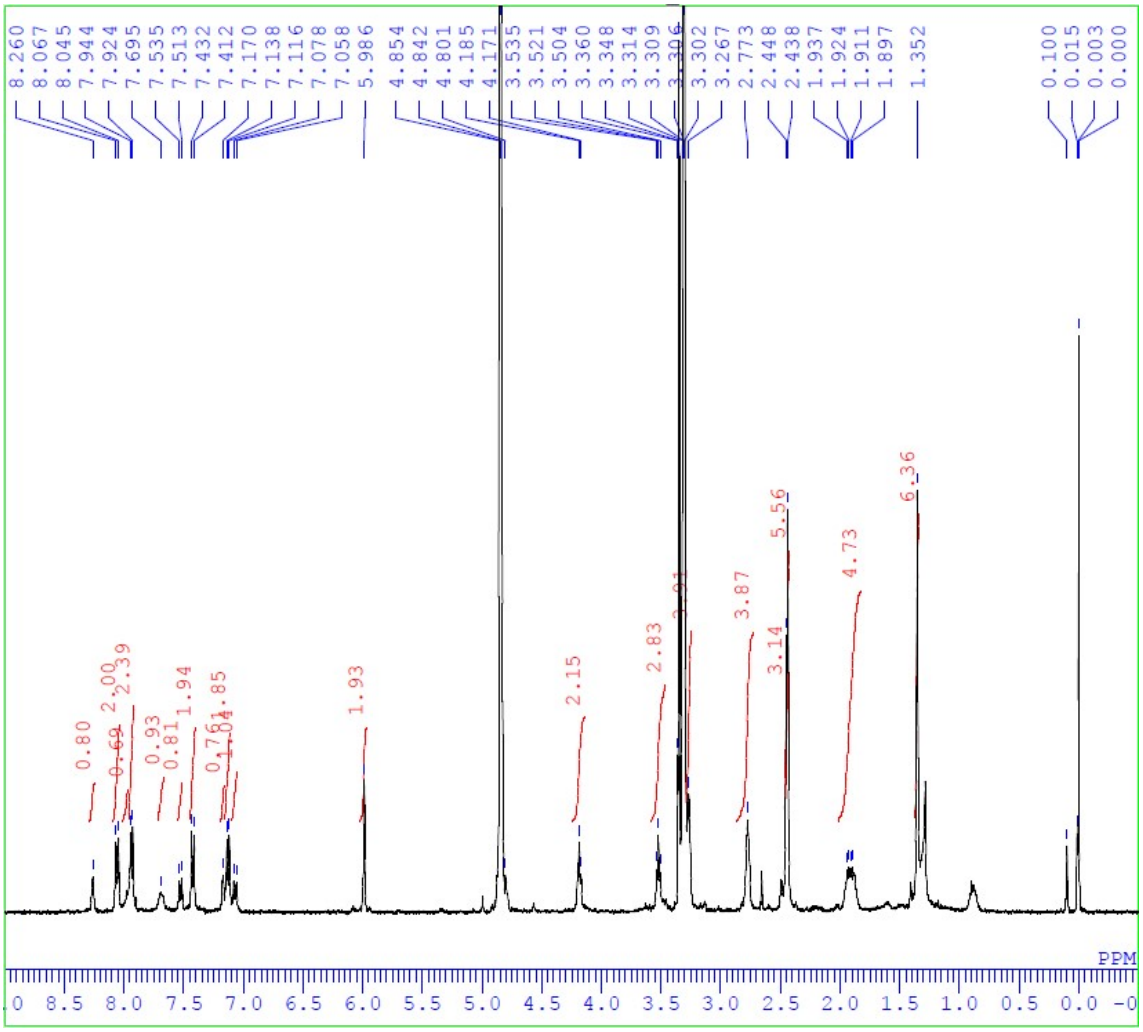
^{1}H NMR spectrum for **BH**



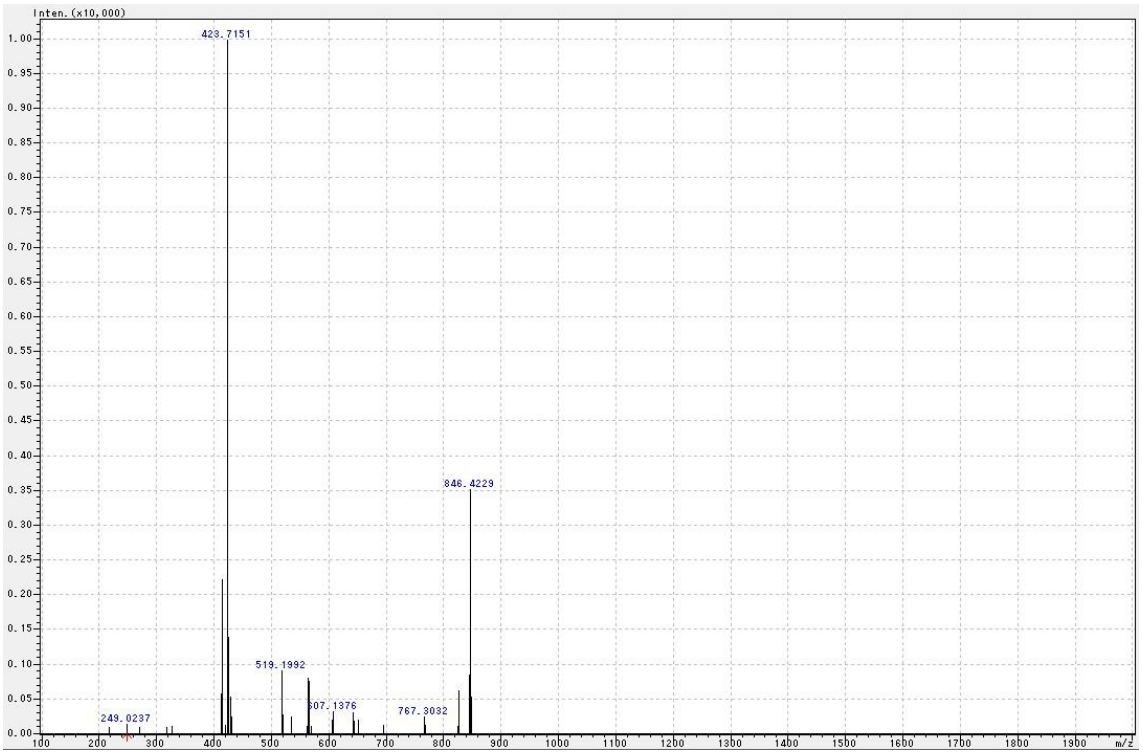
HRMS spectrum for **BH**



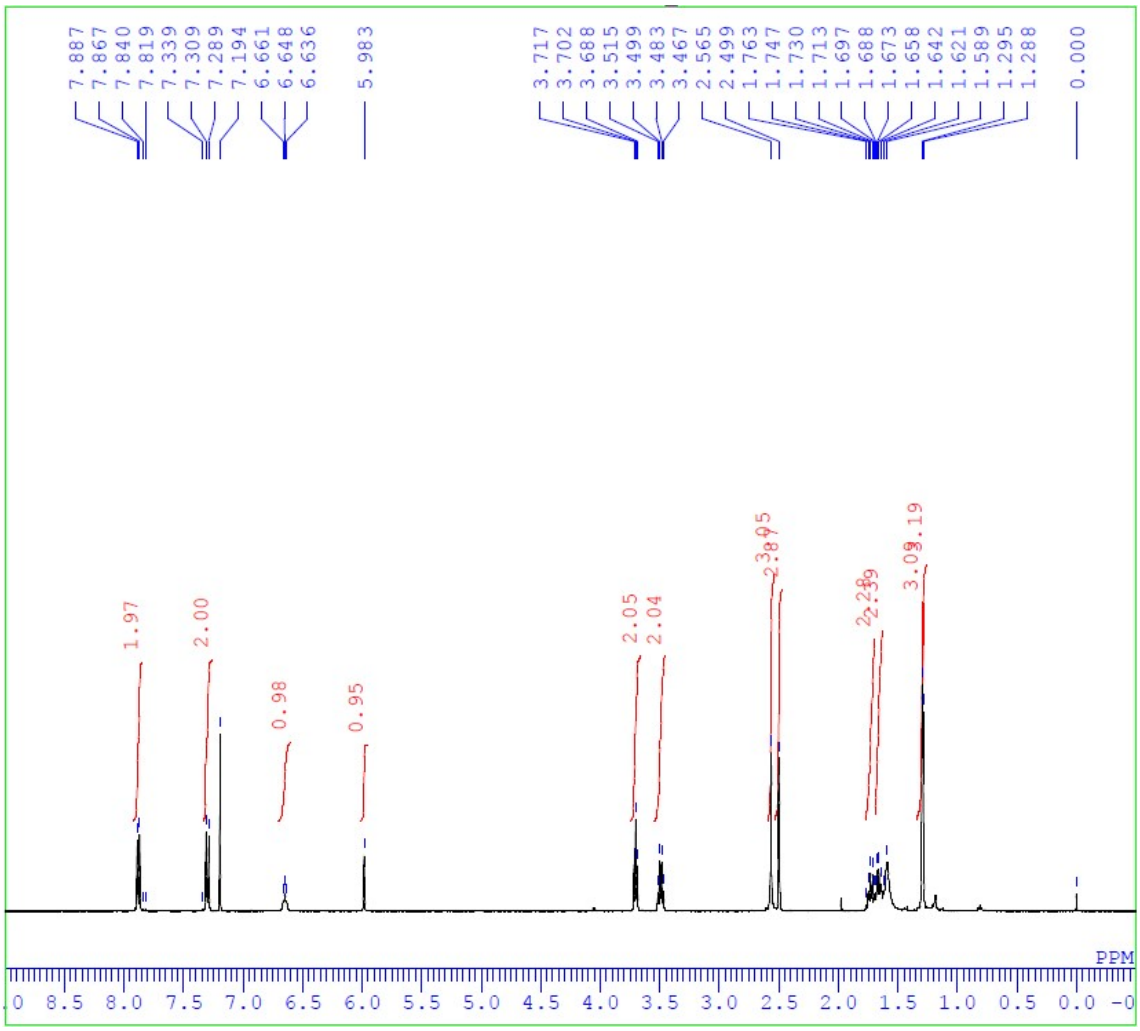
^{1}H NMR spectrum for **pre-BH**



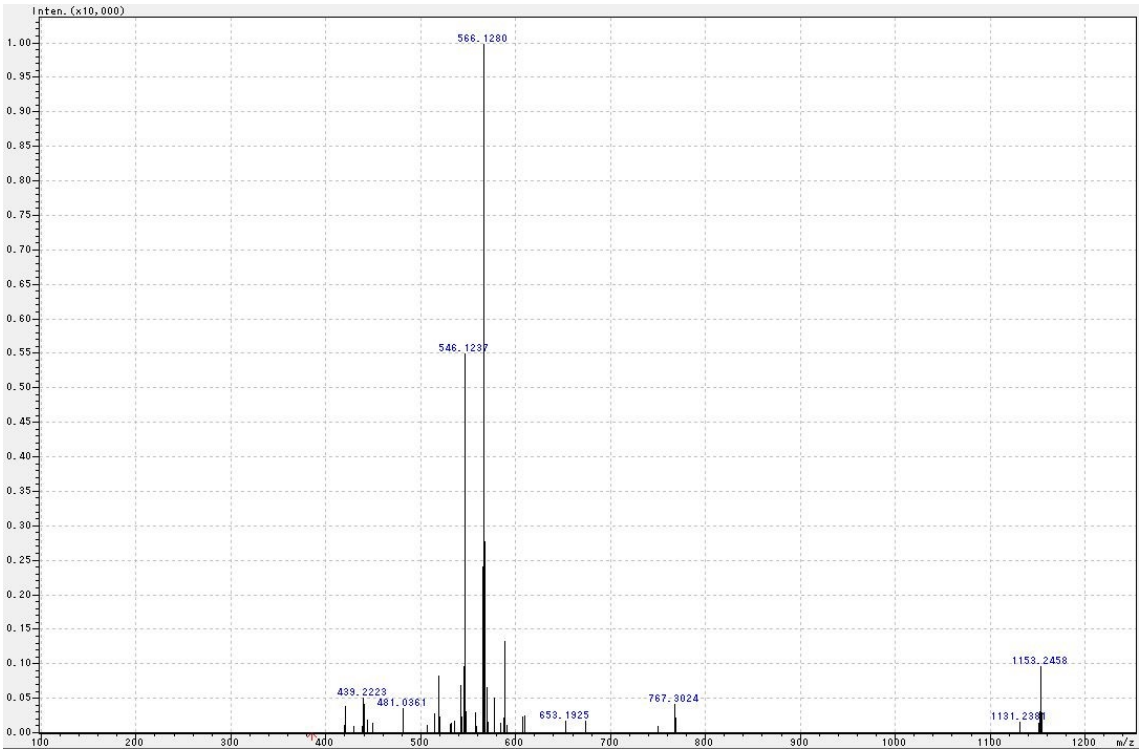
HRMS spectrum for **pre-BH**



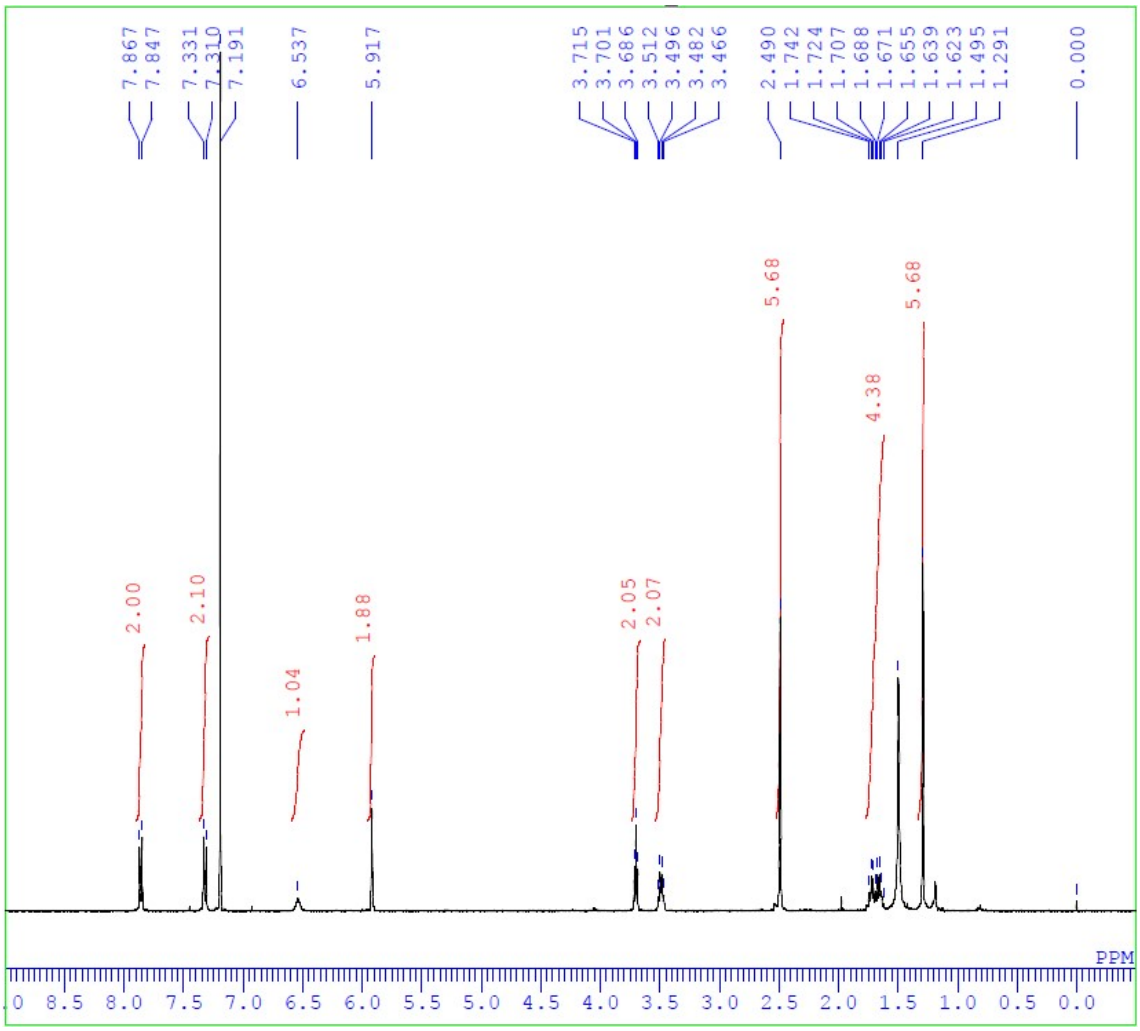
^{1}H NMR spectrum for **BD**



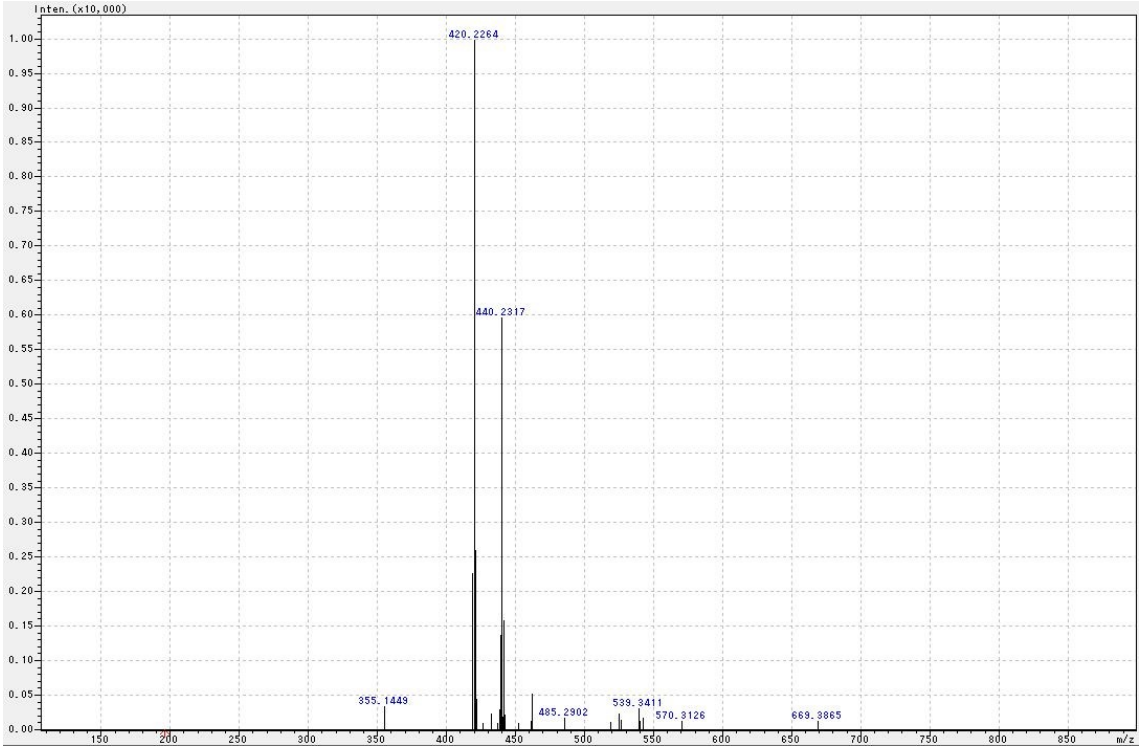
HRMS spectrum for **BD**



^{1}H NMR spectrum for **pre-BD**



HRMS spectrum for **pre-BD**



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