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Supporting Information

Engineering organelle-specific activatable molecules for ultra-fast and reliable in situ mapping of subcellular nitric oxide

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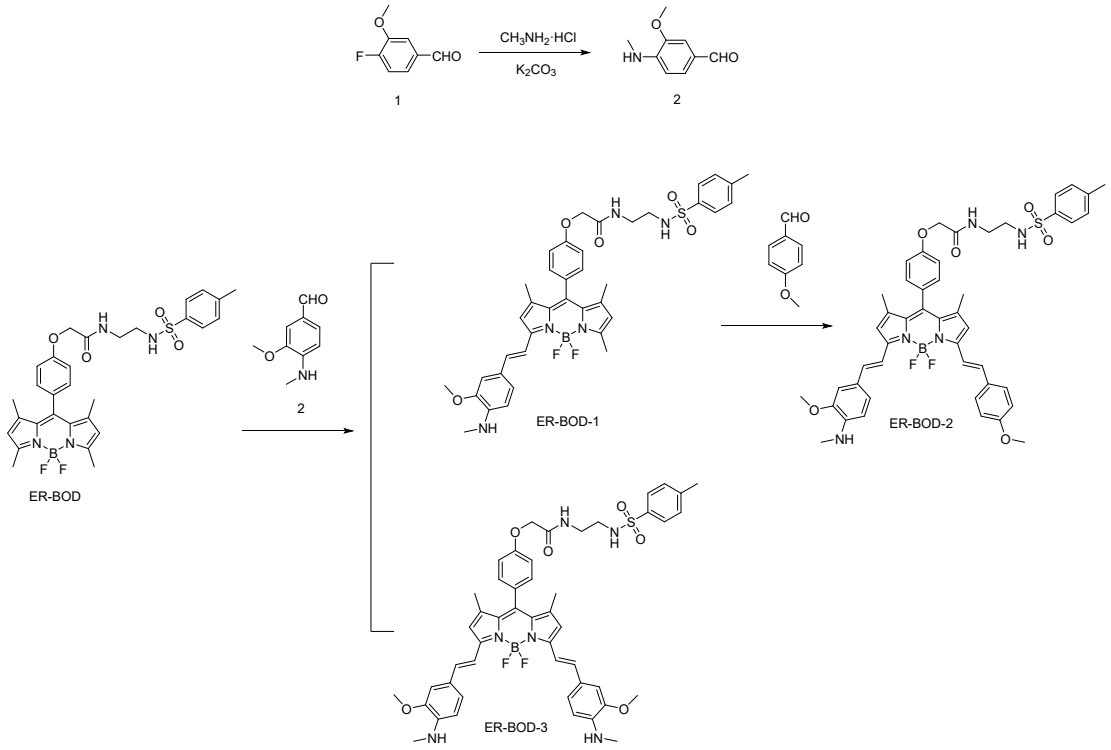
1. Synthesis.



Scheme S1. The synthesis of ER-BOD-X (X=1,2,3).

Synthesis of compound 2.

In a 500 mL flask, 3-methoxy-4-fluorobenzaldehyde (4.62 g, 0.03 mol), potassium carbonate (27.6 g, 0.2 mol), and methylamine hydrochloride (6.7 g, 0.1 mol) were dissolved in an appropriate amount of mixed H2O/DMSO/EtOH (30/75/10, v/v/v) solvent, and the system was heated to 110 °C for 5 days. After the reaction was complete, it was extracted with dichloromethane, washed three times with deionized water and dried with anhydrous sodium sulfate. The solvent was removed by distillation under reduced pressure and separated by column chromatography to give 4.6 g of white solid **compound 2** in 91 % yield. ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 9.62 (s, 1H), 7.41-7.43 (dd, 1H), 7.18-7.19 (d, 1H), 6.56-6.58 (d, 1H), 6.27-6.31 (q, 1H), 3.85 (s, 3H), 2.80 (d, 3H).

Synthesis of compound ER-BOD-1.

The synthesis of ER-BOD was derived from our previous work[1]. ER-BOD (100 mg, 0.17 mmol) and compound 2 (84.2 mg, 0.51 mmol) were weighed and mixed in a 10 ml flask, and 500 μL of piperidine was added to make the mixture fully dissolved, and then the temperature was raised to 100 ℃ and refluxed for 6 hours. The organic phase was extracted with dichloromethane, washed with saturated saline, dried with anhydrous sodium sulfate, and the solvent was removed by distillation under reduced

pressure. 54.2 mg **ER-BOD-1** of blue solid was obtained by silica gel column chromatography in 43% yield. ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 8.18 (t, 1H), 7.69 (t, 3H), 7.50 (d, 1H), 7.38 (d, 2H), 7.29 (d, 2H), 7.21 (d, 1H), 7.13 (t, 3H), 6.96 (d, 2H), 6.54 (d, 1H), 6.10 (s, 1H), 5.85 (d, 1H), 4.50 (s, 2H), 3.86 (s, 3H), 3.17 (m, 2H), 2.78 (m, 5H), 2.46 (s, 3H), 2.36 (s, 3H), 1.42 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (151 MHz, d_6-DMSO, ppm) δ 166.93, 157.39, 154.03, 150.72, 145.78, 142.07, 141.00, 139.09, 137.81, 136.81, 132.21, 130.07, 129.05, 128.95, 126.48, 125.92, 122.66, 119.66, 117.66, 114.72, 111.61, 107.69, 106.93, 66.27, 54.75, 47.99, 41.18, 37.60, 28.78, 20.32, 13.92, 13.41. HRMS (ESI, m/z): calculated for $C_{39}H_{42}BF_2N_5O_5S$ $[M+H]^+$: 742.3041, found: 742.3052.

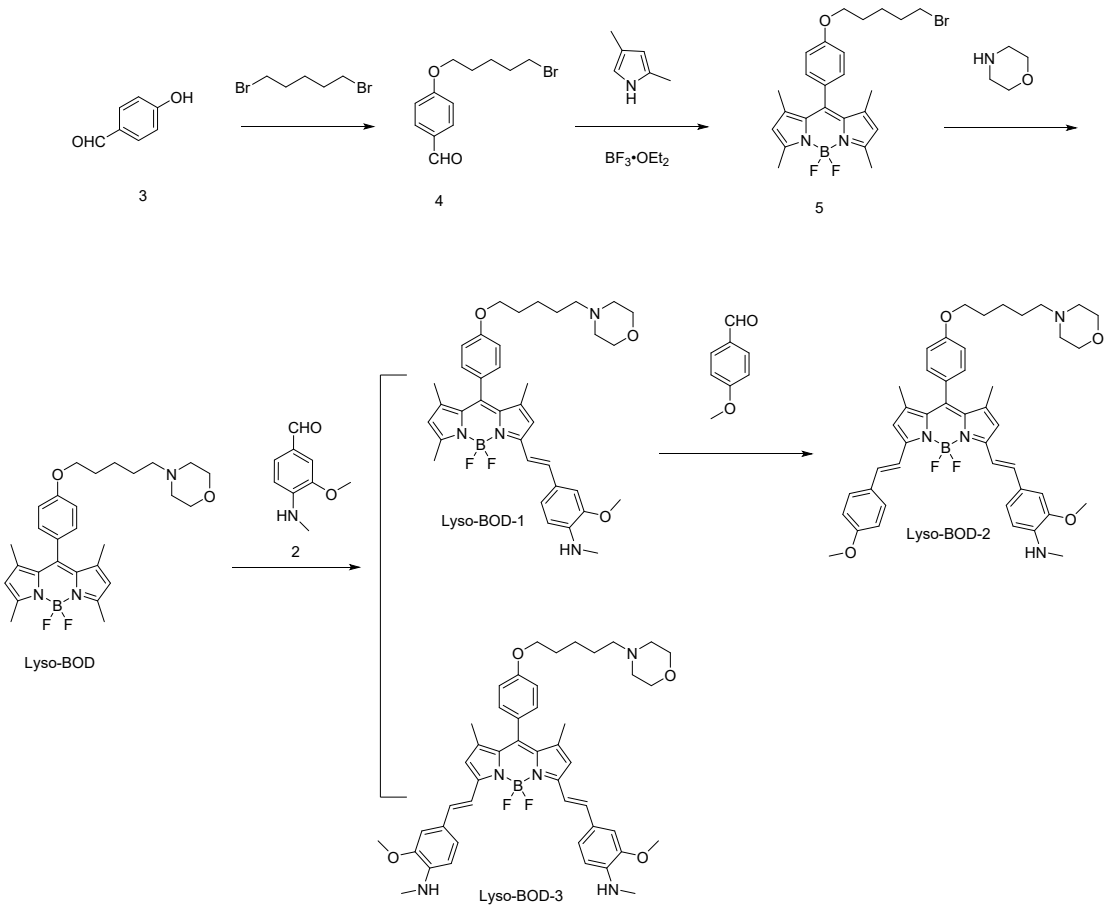
Synthesis of compound ER-BOD-2.

ER-BOD-1 (80 mg, 0.11 mmol) was dissolved in 500 μL of piperidine, to which p-methoxybenzaldehyde (150 mg, 1.10 mmol) was added, and the system was warmed up to 90 ℃. A drop of acetic acid was added, and then refluxed for 3 h. After the reaction was completed and extracted with dichloromethane, the organic phase was washed three times with saturated saline, dried with anhydrous sodium sulfate, and purified by silica gel column chromatography after removing the solvent by distillation under reduced pressure to obtain 48.2 mg blue-green solid **ER-BOD-2** in 53% yield. ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 8.19 (t, 1H), 7.69 (t, 3H), 7.59-7.52 (m, 2H), 7.50 (s, 1H), 7.41 (q, 4H), 7.31 (t, 3H), 7.20 (d, 1H), 7.13 (d, 2H), 7.07-7.00 (m, 3H), 6.89 (d, 2H), 6.56 (d, 1H), 5.87 (q, 1H), 4.50 (s, 2H), 3.89 (s, 3H), 3.81 (s, 3H), 3.21 (q, 2H), 2.91-2.73 (m, 5H), 2.36 (s, 3H), 1.42 (d, 6H). ^{13}C NMR (151 MHz, d_6-DMSO, ppm) δ 168.00, 160.54, 158.46, 154.50, 150.51, 146.83, 143.15, 142.41, 142.08, 140.03, 137.90, 137.02, 135.36, 133.67, 132.68, 130.30, 130.13, 129.54, 129.03, 127.70, 127.00, 123.91, 123.34, 118.83, 117.78, 116.77, 115.75, 115.10, 112.94, 108.87, 108.70, 67.36, 55.95, 55.78, 42.26, 38.68, 29.87, 21.41, 14.99, 14.75. HRMS (ESI, m/z): calculated for $C_{47}H_{48}BF_2N_5O_6S$ $[M+H]^+$: 860.3459, found: 860.3475.

Synthesis of compound ER-BOD-3.

A volume of 500 μL of piperidine was used to dissolve ER-BOD (100 mg, 0.17 mmol) and compound 2 (281 mg, 1.70 mmol), and the temperature was slowly raised to 100 °C. A drop of acetic acid was added and the reaction was refluxed for 4 h. After the reaction was complete, it was extracted using dichloromethane, washed with deionized water, dried with anhydrous sodium sulfate, distilled under reduced pressure to remove the solvent, and separated by column chromatography to obtain 54.3 mg of **ER-BOD-3** as a dark green solid in 36% yield. ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 8.19 (t,

1H), 7.69 (d, 3H), 7.41 (t, 4H), 7.32 (t, 4H), 7.13 (t, 4H), 7.02 (s, 2H), 6.85 (s, 2H), 6.54 (d, 2H), 5.77 (d, 2H), 4.50 (s, 2H), 3.88 (s, 6H), 3.21 (dd, 2H), 2.78 (d, 8H), 2.36 (s, 3H), 1.42 (s, 6H).^{13}C NMR (151 MHz, d_6-DMSO, ppm) δ 170.82, 168.02, 158.39, 152.50, 146.90, 143.15, 141.69, 140.80, 138.16, 137.89, 135.99, 135.99, 132.99, 130.40, 130.13, 127.82, 127.00, 124.14, 123.31, 117.97, 115.69,113.39, 108.77, 107.79, 67.36, 60.23, 55.67, 42.26, 38.68, 29.90, 21.41, 21.23, 14.85, 14.55. HRMS (ESI, m/z): calculated for $C_{48}H_{51}BF_2N_6O_6S$ [M-H]$^-$: 887.3579, found: 887.3580.



Scheme S2. The synthesis of Lyso-BOD-X (X=1,2,3).

Synthesis of compound 4.

In a 250 mL flask, compound 2 (3.05 g, 25 mmol), 1,5-dibromopentane (5.64 mL, 30 mmol) and potassium carbonate (5.18 g, 37.5 mmol) were dissolved in 50 mL acetonitrile, and refluxed overnight at 90 °C. After the reaction, the product was washed with deionized water, extracted with DCM, dried with anhydrous sodium sulfate, the solvent was removed by distillation under reduced pressure and separated by column chromatography to give 5.6 g of a light yellow oily product compound 4 in 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 9.88 (s, 1H), 7.82 (d, 2H), 7.00 (d, 2H), 4.06 (t,

2H), 3.45 (t, 2H), 1.96 (m, 2H), 1.86 (m, 2H), 1.66 (m, 2H).

Synthesis of compound 5.

In a 500 mL flask, 2, 4-dimethylpyrrole (435 mg, 5.0 mmol) was dissolved in 100 mL of dichloromethane in an ice bath, 3 drops of trifluoroacetic acid were added, and compound 4 (677.5 mg, 2.5 mmol) was dissolved in 50 mL of dichloromethane and added to the system, and then the reaction was stirred at room temperature for 6 h. After completion of the reaction, tetra-chloro-benzoquinone (615 mg, 2.5 mmol) was dissolved in 20 mL of dichloromethane and added to the above reaction solution, and the reaction was continued with stirring at room temperature for 2 h. The system was quenched with saturated aqueous sodium bicarbonate, washed with saturated aqueous sodium chloride, extracted with dichloromethane, dried over anhydrous sodium sulfate, and the solvent was removed by distillation under reduced pressure. The crude product was dissolved in 100 mL of toluene, and triethylamine (4.716 mL, 34 mmol) was added under an ice bath with stirring for 20 min at room temperature, followed by the addition of boron trifluoride diethyl etherate (2.38 mL, 34 mmol) under an ice bath with reflux stirring at 90 ℃ for 2 h. After the reaction was completed, the product was washed and dried. The solvent was removed by distillation under reduced pressure, and 390 mg of the orange solid product **compound 5** was separated by column chromatography with a yield of 32 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.15 (d, 2H), 6.99 (d, 2H), 5.97 (s, 2H), 4.02 (t, 2H), 3.46 (t, 2H), 2.55 (s, 6H), 1.97 (m, 2H), 1.86 (m, 2H), 1.68 (m, 2H), 1.43 (s, 6H).

Synthesis of compound Lyso-BOD.

Potassium carbonate (414 mg, 3 mmol) was dissolved in 80 mL acetonitrile, and morpholine (435 μL, 5 mmol) was added dropwise, compound 5 (489 mg, 1 mmol) was dissolved in 20 mL of acetonitrile, and added dropwise to the above solution, and then the reaction was heated up to 50 ℃, refluxed for 2 h. After the reaction was complete, the product was washed by deionized water, extracted by methylene chloride, and dried over anhydrous sodium sulfate. The solvent was removed by spinning under reduced pressure, and 350 mg of orange solid product **Lyso-BOD** was obtained by column chromatography in 71 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.13 (d, 2H), 7.00 (d, 2H), 5.97 (s, 2H), 4.01 (t, 2H), 3.76 (t, 4H), 2.55 (s, 6H), 2.49 (t, 2H), 2.42 (m, 2H), 1.86 (m, 2H), 1.62 (t, 4H), 1.54 (m, 2H), 1.43 (s, 6H).

Synthesis of compound Lyso-BOD-1.

The synthesis process of **Lyso-BOD-1** was same as **ER-BOD-1** with a yield of 59%. ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 7.47 (d, 1H), 7.25 (d, 2H), 7.18 (d, 1H), 7.13

(d, 1H), 7.08 (d, 2H), 6.98 (s, 1H), 6.90 (s, 1H), 6.55 (d, 1H), 6.11 (s, 1H), 5.83 (m, 1H), 4.03 (t, 2H), 3.86 (s, 3H), 3.56 (m, 4H), 2.79 (d, 3H), 2.46 (s, 3H), 2.33 (s, 4H), 2.28 (m, 2H), 1.76 (m, 2H), 1.47 (m, 4H), 1.45 (s, 3H), 1.39 (s, 3H). ^{13}C NMR (151 MHz, *d*$_6$-DMSO, ppm) δ 159.64, 155.03, 151.74, 146.85, 143.13, 142.06, 140.18, 140.09, 139.15, 133.33, 131.21, 129.96, 126.67, 123.73, 123.67, 120.71, 118.69, 115.46, 112.70, 108.75, 107.99, 68.02, 66.66, 58.63, 55.82, 53.83, 40.52, 29.86, 29.01, 26.81, 26.13, 23.91, 14.98, 14.71, 14.48. HRMS (ESI, m/z): calculated for $C_{37}H_{45}BF_2N_4O_3$ $[M+H]^+$: 643.3626, found: 643.3638.

Synthesis of compound Lyso-BOD-2.

The synthesis process of **Lyso-BOD-2** was same as **ER-BOD-2** with a yield of 48%. ^{1}H NMR (400 MHz, *d*$_6$-DMSO, ppm) δ 7.54 (d, 2H), 7.49 (s, 1H), 7.42 (d, 2H), 7.28 (d, 3H), 7.19 (d, 1H), 7.09 (d, 2H), 7.02 (d, 3H), 6.93 (s, 1H), 6.86 (s, 1H), 6.55 (s, 1H), 5.86 (s, 1H), 4.04 (t, 2H), 3.89 (s, 3H), 3.81 (s, 3H), 3.57 (t, 4H), 2.80 (d, 3H), 2.33 (s, 4H), 2.28 (m, 2H), 1.77 (m, 2H), 1.47 (m, 4H), 1.46 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (151 MHz, *d*$_6$-DMSO, ppm) δ 160.52, 159.64, 154.44, 150.47, 146.83, 142.42, 142.05, 140.05, 139.94, 137.28, 135.31, 133.72, 132.75, 132.29, 130.23, 129.55, 129.02, 126.75, 123.91, 123.30, 118.80, 117.69, 116.77, 115.42, 115.09, 114.99, 112.96, 108.86, 108.69, 68.04, 66.68, 58.65, 56.16, 55.95, 55.78, 53.84, 40.52, 29.87, 29.02, 26.15, 23.93, 14.98, 14.74. HRMS (ESI, m/z): calculated for $C_{45}H_{51}BF_2N_4O_4$ $[M+H]^+$: 761.4044, found: 761.4056.

Synthesis of compound Lyso-BOD-3.

The synthesis process of **Lyso-BOD-3** was same as **ER-BOD-3** with a yield of 42%. ^{1}H NMR (400 MHz, *d*$_6$-DMSO, ppm) δ 7.39 (d, 2H), 7.28 (m, 4H), 7.11 (m, 4H), 7.01 (s, 2H), 6.85 (s, 2H), 6.53 (d, 2H), 5.78 (s, 2H), 4.02 (t, 2H), 3.88 (s, 6H), 3.57 (t, 4H), 2.78 (d, 6H), 2.33 (s, 4H), 2.28 (m, 2H), 1.77 (m, 2H), 1.48 (m, 4H), 1.45 (s, 6H). ^{13}C NMR (151 MHz, *d*$_6$-DMSO, ppm) δ 159.00, 154.29, 146.32, 141.11, 140.24, 137.60, 137.55, 132.48, 129.77, 123.56, 122.71, 119.12, 114.80, 112.84, 108.20, 107.21, 106.56, 66.12, 59.65, 55.10, 54.81, 53.28, 39.94, 29.34, 28.44, 23.34, 20.66, 14.28, 13.98. HRMS (ESI, m/z): calculated for $C_{46}H_{54}BF_2N_5O_4$ $[M+H]^+$: 790.4310, found: 790.4336.

2. Cells culture and imaging.

HeLa cells and RAW 264.7 cells were cultured at 37 °C in a humidified atmosphere of 5/95 CO_2/air incubator within Dulbecco's Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). These cells were seeded in glass bottom dishes and allowed to adhere for 24 h prior to experiments.

For ER and lysosomal co-localization, HeLa cells were pretreated with 100 μM DEA·NONOate for 30 min and then incubated with ER-BOD-1 or Lyso-BOD-1 (5 μM) for another 30 min, rinsed with PBS, and then incubated with 1 μM ER Tracker Green or Lyso-Sensor Green for 20 min, respectively. The confocal imaging was performed using Leica TCS SP8 with a 63 x oil objective. The excitation wavelengths of the probe and Tracker were 540 nm and 443 nm, and the fluorescence signals were collected at 550-650 nm and 490-520 nm.

For visualization of cellular NO, HeLa cells were divided into three groups and the cells were incubated with 0 μM, 50 μM and 100 μM DEA·NONOate for 30 min, followed by the addition of the probes ER-BOD-1 or Lyso-BOD-1 to incubate another 30 min, washed and then imaged using a confocal microscope; RAW 264.7 cells were divided into four groups, and the cells were incubated with ER-BOD-1 or Lyso-BOD-1 (5 μM) for 5 min,10 min,20 min,30 min, respectively, and the cells were washed and imaged using a confocal microscope. The excitation wavelength of ER-BOD-1 and Lyso-BOD-1 was 540 nm, and 550-650 nm was collected for the fluorescence signals.

For activator and inhibitor assays, HeLa cells were pretreated with (1) 0.5 mM PTIO for 30 min, (2) 20 μg/mL LPS for 12 h, (3) 20 μg/mL LPS and 10 μM L-NNA for 12 h, further incubated with ER-BOD-1 or Lyso-BOD-1 (5 μM) for 30 min. The cells were washed and imaged using a confocal microscope. The excitation wavelength of ER-BOD-1 and Lyso-BOD-1 was 540 nm, and 550-650 nm was collected for the fluorescence signals.

3. Cytotoxicity in vitro.

Hela cells were seeded in 96-well microplates in DMEM supplemented with 10% FBS at 37 °C in humidified environment of 5% CO2. After 12 h of cell attachment, the plates were washed with PBS, followed by addition of various concentrations of probe ER-BOD-1 or Lyso-BOD-1 (0-25 μM) in DMEM. The cells were then incubated at 37 °C in humidified environment of 5% CO_2 for 24 h, followed by standard CCK-8 assay.

4. Fluorescence titration.

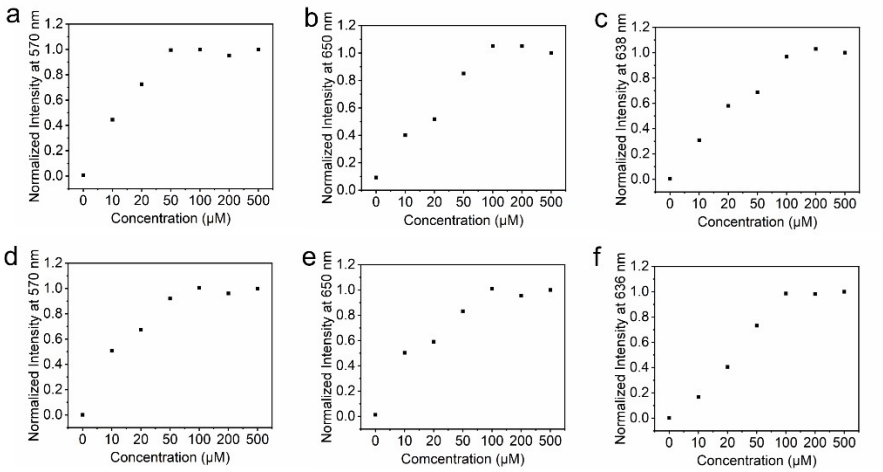


Figure S1. Fluorescence titration experiments of all probes with different concentrations of DEA·NONOate. (a)ER-BOD-1, (b)ER-BOD-2, (c)ER-BOD-3, (d)Lyso-BOD-1, (e)Lyso-BOD-2 and (f)Lyso-BOD-3.

5. HRMS demonstration of ER-BOD-1 towards NO.

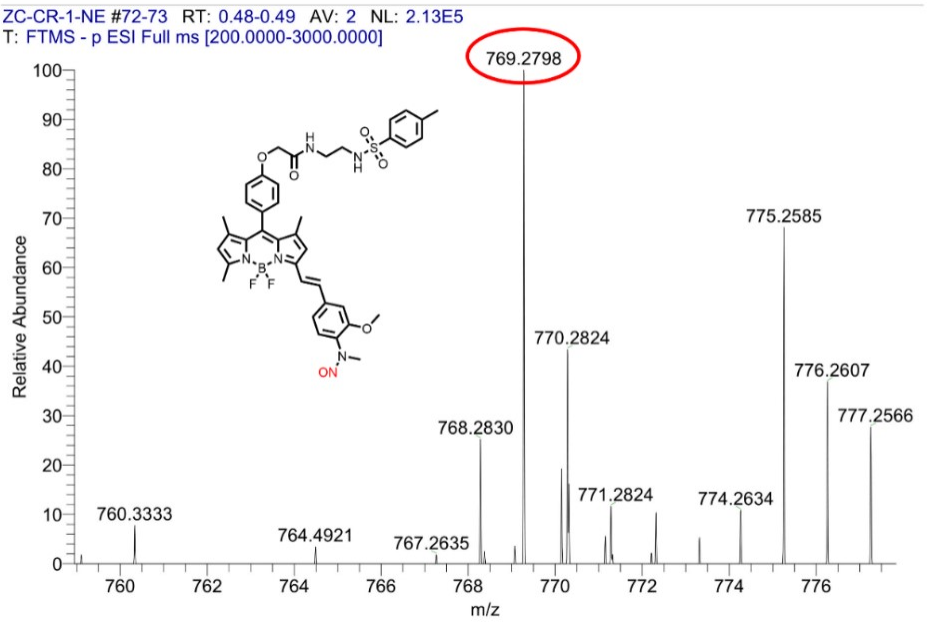


Figure S2. HRMS demonstration of the production from ER-BOD-1 + NO.

6. DFT calculations for PET process.

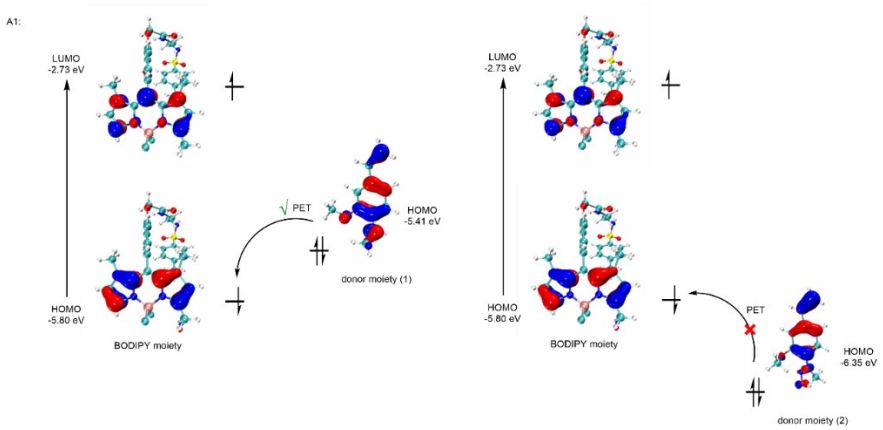
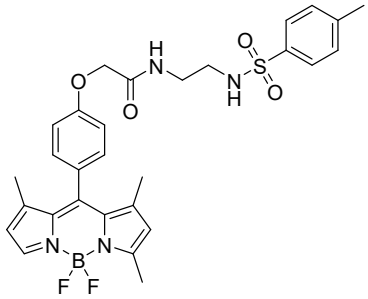


Figure S3. Frontier orbital energy representation of the PET processes in ER-BOD-1. All calculations were performed using Gaussian 16 software. The B3LYP function was used for all calculations combined with the D3BJ dispersion correction. For geometry optimization, the 6-31+G(d,p) basis set was used for all atoms. Resonance frequency calculations were performed at the same level of theory for all stationary points to verify that they are local minima and to derive thermal corrections. The frontier molecular orbital (FMO) analysis was visualized with Multiwfn. All optimized structures were generated using CYLview.



	X	Y	Z
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C	4.29683900	2.04940400	1.87986500
C	5.31554900	0.04505700	-2.65053900
C	4.70144100	-1.06068600	-3.25119300
C	3.70345000	-1.52319100	-2.38022200
C	3.73459900	-0.65825500	-1.24414600
N	4.74405500	0.28897300	-1.45154300
H	6.12807500	0.65895300	-3.01524100
H	4.95778400	-1.48614800	-4.21184500

N	4.28447700	1.24491300	0.79128300
C	1.45054500	-0.15062700	2.83009400
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H	1.09699800	0.42638300	3.68854900
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H	3.19559200	-3.25499600	-3.50978100
H	2.78918100	-3.38705000	-1.78622300
H	1.79112000	-2.40424600	-2.84830700
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C	2.07241300	-2.74574900	0.97797300
C	0.67294700	-1.45333800	-0.50121200
C	1.03598100	-3.65210700	1.17087000
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H	-1.31728700	-2.16754600	-0.81914600
B	5.17135600	1.38163600	-0.46072900
C	3.28305600	1.63525900	2.76834700
H	3.05832800	2.10725700	3.71536700
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H	-3.38543500	1.01241100	-2.29921800
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H	-0.97286600	1.09752700	-1.74186000
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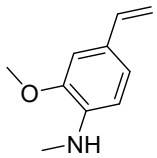
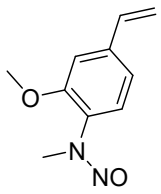
H	0.39456800	2.32903400	1.49474600
H	0.77463000	1.42872600	0.01268100
H	0.62615100	3.18599100	-0.03143600
C	5.24563900	3.18328700	2.05318700
H	6.28418400	2.84214400	1.99444700
H	5.10486500	3.93702000	1.27140200
H	5.08447000	3.65367800	3.02487100



	X	Y	Z
C	-0.64535500	0.48932100	-0.11460200
C	-0.93784900	-0.89124500	-0.22134700
C	0.14263100	-1.78688800	-0.21610300
C	1.46184100	-1.35617200	-0.10377600
C	1.75013900	0.01110400	0.02911700
C	0.67418200	0.92040800	0.02330300
H	-0.07293500	-2.84929700	-0.30007900
H	2.26003800	-2.09099200	-0.10226900
H	0.89175000	1.97820000	0.11041100
O	-1.71693700	1.34699500	-0.18750700
C	-1.46456900	2.75301900	-0.08856700
H	-0.81572900	3.09498100	-0.90198500
H	-2.44028600	3.23239200	-0.17181000
H	-1.01207100	3.00373900	0.87680300
N	-2.25659500	-1.36148200	-0.39388600
H	-2.22614900	-2.36945700	-0.51575000
C	-3.23237400	-1.04452600	0.66493400
H	-4.14633600	-1.60811600	0.46583000
H	-2.86468000	-1.31568700	1.66467800

H	-3.47351700	0.01748900	0.66113800
C	3.11374400	0.54317200	0.16871800
C	4.26314100	-0.15266300	0.16714200
H	3.16715600	1.62543200	0.28299900
H	5.21586200	0.35570500	0.28011300
H	4.29538700	-1.23314600	0.05427200



	X	Y	Z
C	0.03081000	0.83639700	-0.19800800
C	-0.44612300	-0.47243700	-0.03025300
C	0.46253700	-1.52540300	0.12132800
C	1.83055000	-1.28659100	0.08979800
C	2.32661700	0.01806100	-0.09959900
C	1.40437600	1.06752500	-0.23829800
H	0.08445800	-2.53372200	0.25554000
H	2.51300000	-2.12148200	0.20191200
H	1.75154500	2.08774800	-0.36957100
O	-0.83502900	1.90382300	-0.34289100
C	-1.39904300	2.36784800	0.90350600
H	-0.60248800	2.71424400	1.57028800
H	-2.05995200	3.19681800	0.64813000
H	-1.97260400	1.57450100	1.39293300
N	-1.84899100	-0.74066900	0.01066800
N	-2.55436000	-0.35251400	-1.02696000
C	-2.48875000	-1.35016400	1.18242700
H	-1.71717200	-1.54426300	1.92338800
H	-3.23415200	-0.66115400	1.58833900
H	-2.98448100	-2.28072600	0.89541100
O	-3.78590600	-0.54490600	-0.92252700
C	3.76341000	0.33400300	-0.15111100
C	4.77971500	-0.53180000	-0.01803800
H	3.99156800	1.38598000	-0.31421800
H	5.80754100	-0.18674900	-0.07383500
H	4.62908000	-1.59488600	0.14855900

7. The selective responsiveness of ER-BOD-X (X=1,2,3).

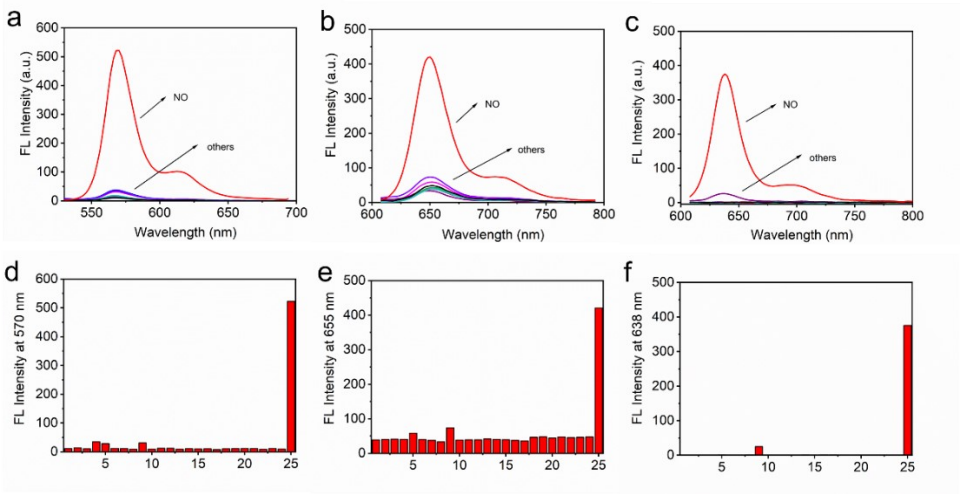


Figure S4. Fluorescence intensity changes of ER-BOD-X (X=1,2,3) upon addition of DEA·NONOate (100 μM) and other biologically relevant analytes (1 mM). 1) Free; 2) NO_2^-; 3) NO_3^-; 4) ·OH; 5) ClO^-; 6) H_2O_2; 7) O_2^-; 8) $^1O_2$; 9) $ONOO^-$; 10) GSH; 11) Cys; 12) Hcy; 13) L-AA; 14) DHA; 15) SO_3^{2-}; 16) SO_4^{2-}; 17) HS^-; 18) $S_2O_4^{2-}$; 19) Na^+; 20) K^+; 21) Ca^{2+}; 22) Fe^{2+}; 23) Fe^{3+}; 24) Cu^{2+}; 25) DEA·NONOate. Data were recorded 60 s (ER-BOD-1 and ER-BOD-2) or 120 s (ER-BOD-3) after addition of analytes. (a, d): ER-BOD-1; (b, e): ER-BOD-2; (c, f): ER-BOD-3.

8. Dose-dependent relationship of ER-BOD-X (X=1,2,3).

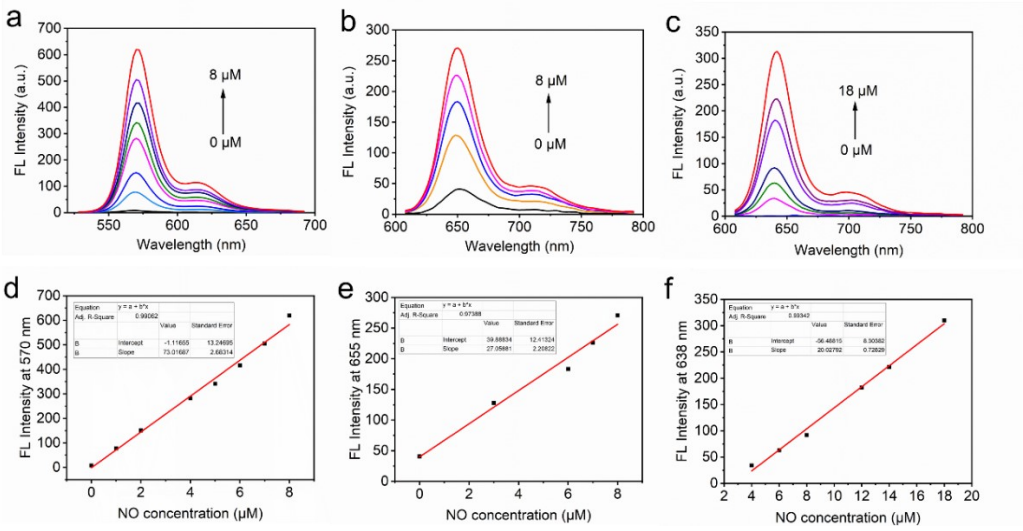


Figure S5. (a), (b), (c) The fluorescence spectra of different probes in the presence of various concentrations of DEA·NONOate. (d), (e), (f) Linear correlation between fluorescence intensity changes of different probes and the NO concentration. (a, d): ER-BOD-1; (b, e): ER-BOD-2; (c, f): ER-BOD-3.

9. pH stability.

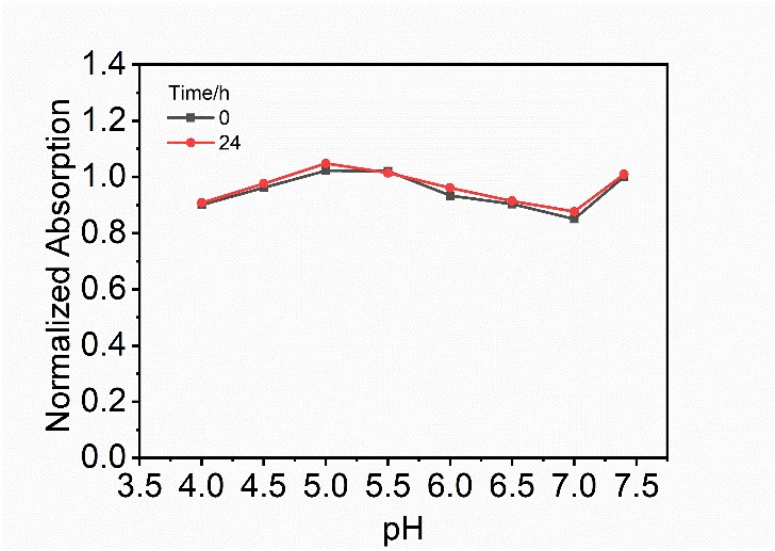


Figure S6. In the various acidic physiological pH, the normalized absorption changes of Lyso-BOD-1 at 600 nm.

10. HRMS demonstration of Lyso-BOD-1 towards NO.

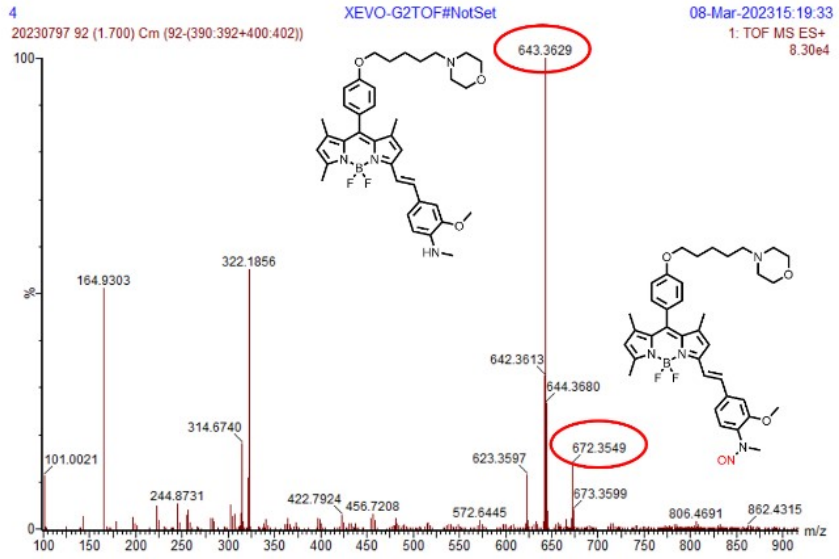


Figure S7. HRMS demonstration of the production from Lyso-BOD-1 + NO.

11. DFT calculations for PET process.

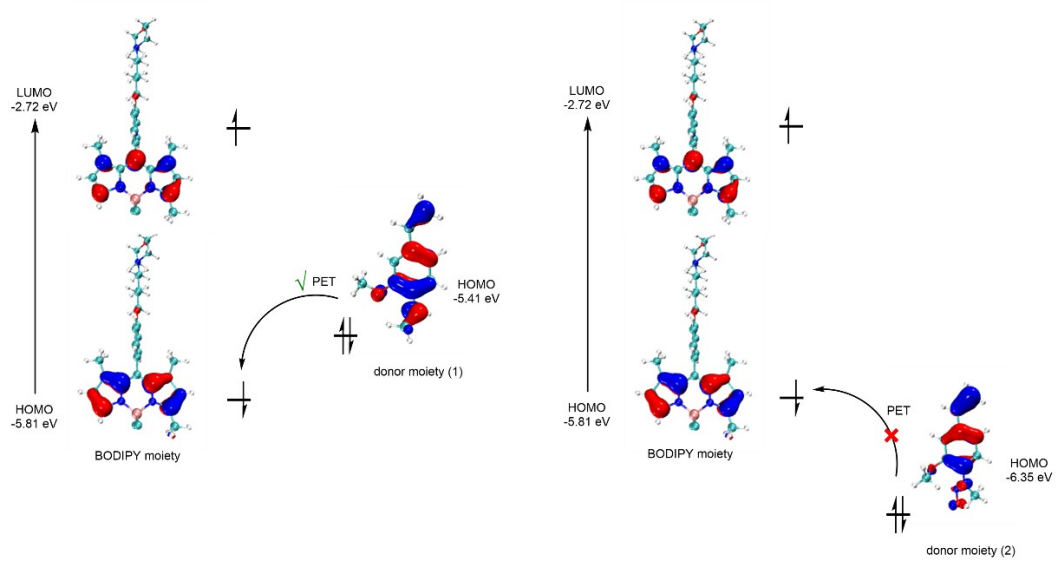
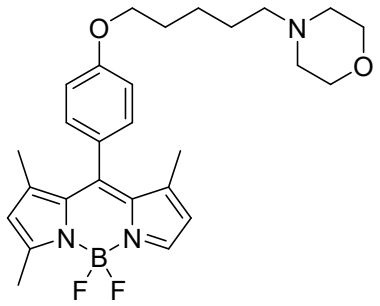


Figure S8. Frontier orbital energy representation of the PET processes in Lyso-BOD-1.



	X	Y	Z
C	4.63610900	1.37734100	0.03500600
C	3.92384800	0.16994400	-0.09579200
C	4.21309900	2.74162500	0.02549800
C	6.45011400	2.63160200	0.36477200
H	7.49477000	2.85263700	0.53777000
C	6.33875900	-2.42425700	0.26976700
C	5.20363800	-3.23191200	0.04876200
C	4.09658900	-2.40424800	-0.12469200
C	4.57970600	-1.05957400	0.00096900
N	5.96671400	-1.12337000	0.23973500
H	5.20897600	-4.31316700	0.01650500
N	6.01934400	1.35697100	0.24999000
C	2.83493400	3.29871800	-0.15278300
H	2.11441000	2.82970400	0.52319100
H	2.84616200	4.37427700	0.04232200

H	2.46377400	3.14163300	-1.17052500
C	2.70462300	-2.88182700	-0.39430300
H	2.71614000	-3.95990200	-0.57426800
H	2.03979600	-2.68357800	0.45214400
H	2.26555900	-2.38631400	-1.26469800
C	2.45772100	0.21096400	-0.33884000
C	1.55788000	0.02438200	0.71189100
C	1.95960100	0.44492700	-1.62848300
C	0.17812000	0.07065000	0.49639000
H	1.93287100	-0.15744800	1.71416200
C	0.58995200	0.48205000	-1.86059400
H	2.64964200	0.59461300	-2.45278000
C	-0.30748800	0.29703600	-0.79768800
H	-0.49524200	-0.07209400	1.33169300
H	0.19969700	0.65600400	-2.85817700
B	6.89436100	0.10022100	0.36458300
C	5.36806700	3.51058200	0.23172500
H	5.41563500	4.58967700	0.28516900
F	7.57714900	0.09039400	1.60769000
F	7.87410800	0.09136400	-0.66301300
O	-1.63799700	0.35503100	-1.12050300
C	-2.60723300	0.16505900	-0.06892800
C	-3.98994700	0.27839300	-0.68300700
H	-2.45701600	0.93049200	0.70160200
H	-2.45730200	-0.82241100	0.38395800
C	-5.08964800	0.08465800	0.36492400
H	-4.09432700	1.26431100	-1.15278900
H	-4.09488600	-0.47355300	-1.47491800
C	-6.49566100	0.21571800	-0.22867900
H	-4.97994200	-0.90460500	0.82925400
H	-4.96326100	0.82336100	1.16788700
H	-6.61267000	1.21260400	-0.67261200
H	-6.60578000	-0.51175100	-1.04121200
C	-7.57395300	0.00266500	0.83107600
H	-7.47490800	-1.01629000	1.25121000
H	-7.40183400	0.70223100	1.65678300
C	-9.34962100	-0.80169800	-0.61953700
C	-9.87442300	0.15496000	1.49886300
C	-10.79634500	-0.61055600	-1.04414300
H	-9.22582000	-1.81468100	-0.19298800
H	-8.71821500	-0.73511000	-1.50862500
C	-11.31311200	0.34019900	1.04817800
H	-9.78358300	-0.81628000	2.01984500
H	-9.61931900	0.94213100	2.21548700

H	-11.10480400	-1.41508500	-1.71570500
H	-10.91861400	0.35195600	-1.56176600
H	-11.99633000	0.22577800	1.89291000
H	-11.45286300	1.33881500	0.60941600
N	-8.94933200	0.22237800	0.35768300
O	-11.68600700	-0.65482800	0.08204400
C	7.73891100	-2.87293500	0.50349600
H	8.09148600	-2.55968000	1.49194300
H	7.79295000	-3.96139500	0.44274800
H	8.42127000	-2.44408500	-0.23721400

12. Dose-dependent relationship of Lyso-BOD-X (X=1,2,3).

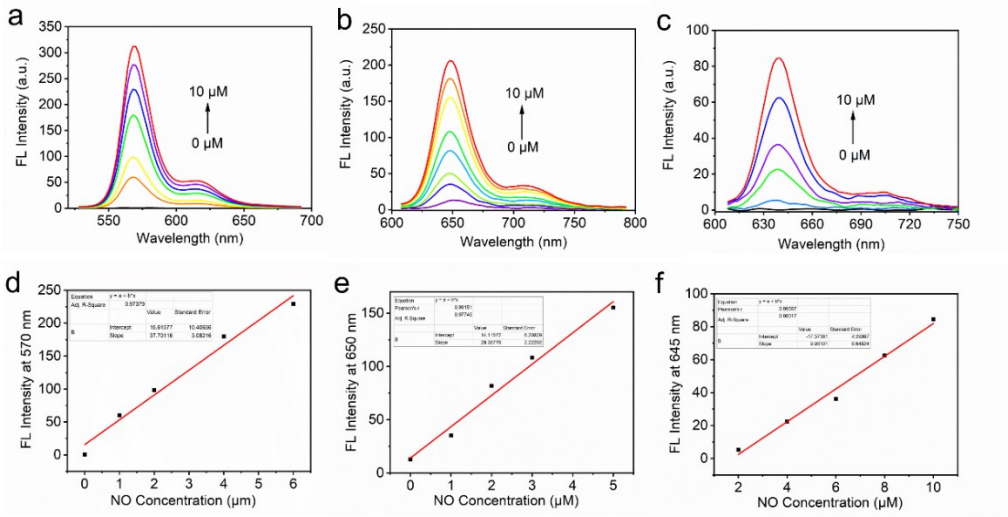


Figure S9. (a), (b), (c) The fluorescence spectra of different probes in the presence of various concentrations of DEA·NONOate. (d), (e), (f) Linear correlation between fluorescence intensity changes of different probes and the NO concentration. (a, d): Lyso-BOD-1; (b, e): Lyso-BOD-2; (c, f): Lyso-BOD-3.

13. The selective responsiveness of Lyso-BOD-X (X=1,2,3).

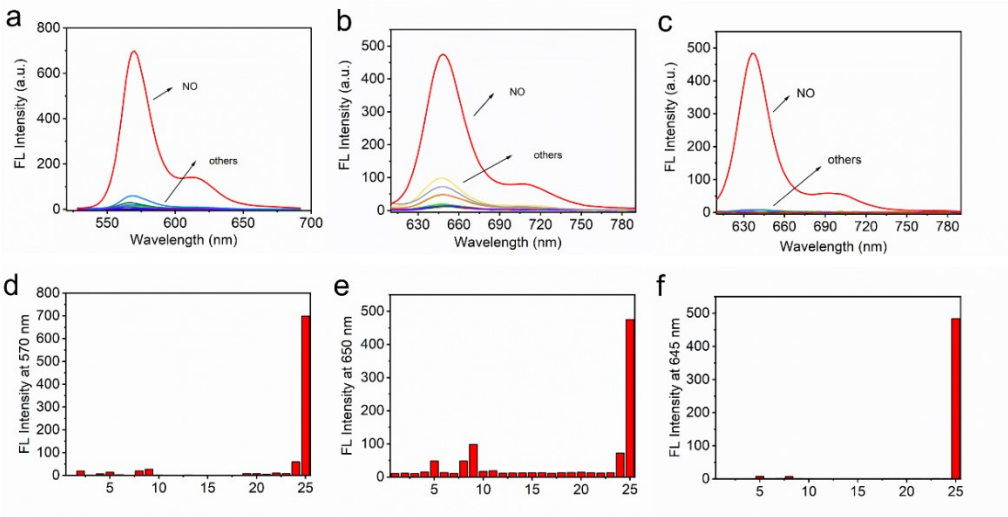


Figure S10. Fluorescence intensity changes of Lyso-BOD-X (X=1,2,3) upon addition of DEA·NONOate (100 μM) and other biologically relevant analytes (1 mM). 1) Free; 2) NO_2^-; 3) NO_3^-; 4) ·OH; 5) ClO^-; 6) H_2O_2; 7) O_2^-; 8) $^1O_2$; 9) $ONOO^-$; 10) GSH; 11) Cys; 12) Hcy; 13) L-AA; 14) DHA; 15) SO_3^{2-}; 16) SO_4^{2-}; 17) HS^-; 18) $S_2O_4^{2-}$; 19) Na^+; 20) K^+; 21) Ca^{2+}; 22) Fe^{2+}; 23) Fe^{3+}; 24) Cu^{2+}; 25) DEA·NONOate. Data were recorded 60 s (Lyso-BOD-1 and Lyso-BOD-2) or 120 s (Lyso-BOD-3) after addition of analytes. (a, d): Lyso-BOD-1; (b, e): Lyso-BOD-2; (c, f): Lyso-BOD-3.

14. The stability of ER-BOD-1 and Lyso-BOD-2 against GSH.

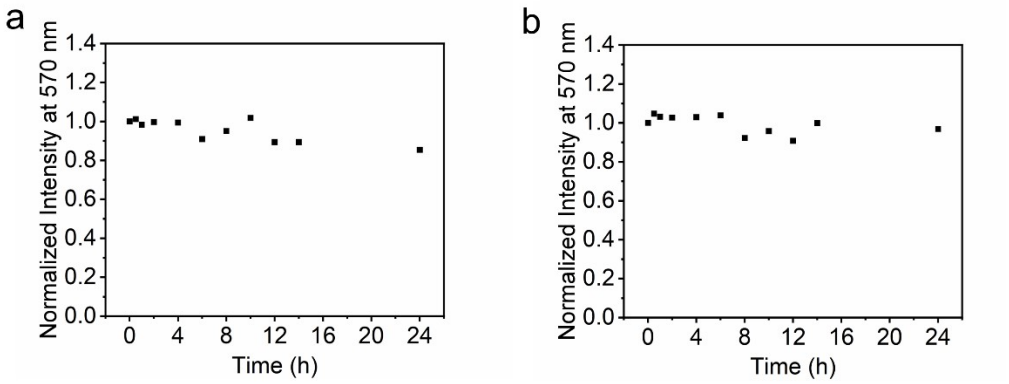
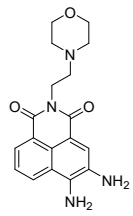
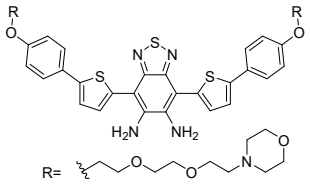
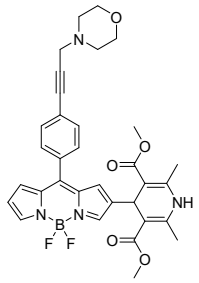
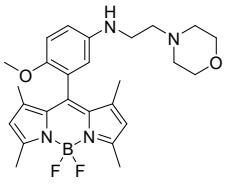


Figure S11. The normalized fluorescence changes of the products at 570 nm against 10 mM GSH at the end of the reaction of (a) ER-BOD-1 or (b) Lyso-BOD-1 with NO.

15. Summary of fluorescent probes.

Probe	Response time	LOD	Ref.
	25 min	4.57 μM	*Chin Chem Lett.*, 2016, **27**, 1554–1558
	30 min	242 nM	*Anal. Chem.*, 2021, **93**, 4975-4983
	15 min	47 nM	*Sensors and Actuators B: Chemical*, 2021, **339**, 129880
	20 min	18.7 nM	*Anal. Chim. Acta.*, 2019,**1067**, 88-97

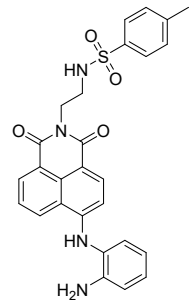
	6 min	3.3 nM	*ACS Sens.*, 2018, **3**, 2311−2319
ER-BOD-1	60 s	4.5 nM	This work
Ly-BOD-1	60 s	9.3 nM	This work

Table S1. Summary of fluorescent probes with ER or lysosomal targeting capabilities for the detection of NO.

16. The cytotoxicity.

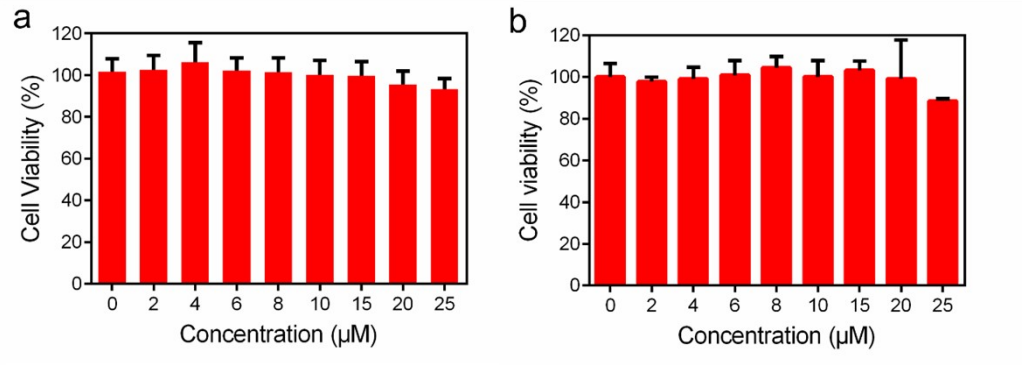


Figure S12. The cytotoxicity of (a) ER-BOD-1 and (b) Lyso-BOD-1 to living HeLa cells.

17. The distribution morphology.

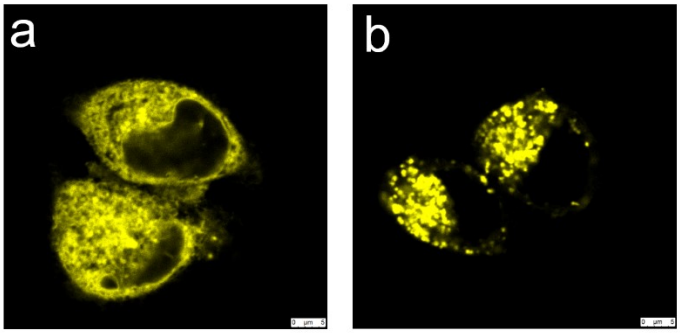


Figure S13. Distribution morphology of the probes in the cell: Confocal fluorescence images of HeLa cells co-stained with (a) ER-BOD-1 and ER-Tracker Green, (b) Lyso-BOD-1 and Lyso-Sensor Green. Scale bars: 5 μm.

18. Colocalization experiments.

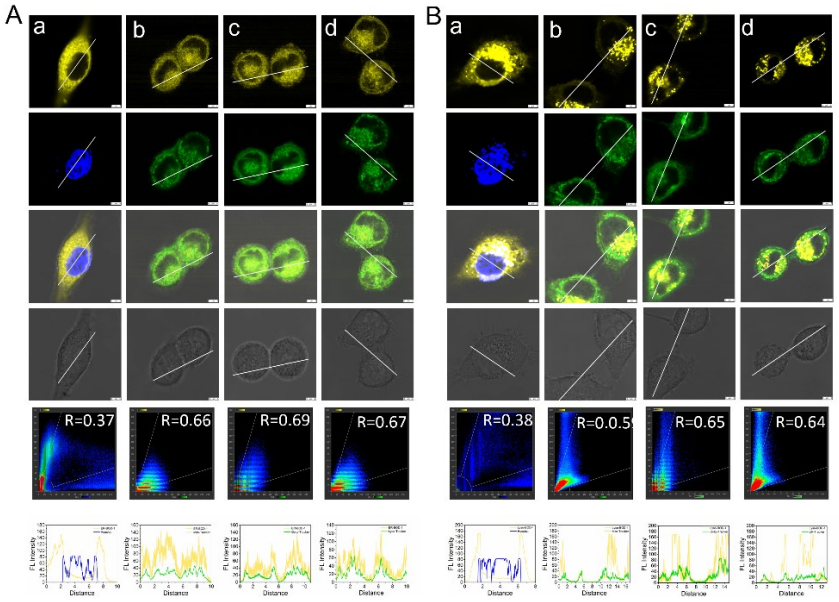


Figure S14. Colocalization experiments of the probes with other organelles. (A) ER-BOD-1 with (a) Hoechst 33342, (b) Mito-Tracker Green, (c) Golgi-Tracker Green and (d) Lyso-Sensor Green; (B) Ly-BOD-1 with (a) Hoechst 33342, (b) Mito-Tracker Green, (c) Golgi-Tracker Green and (d) ER-Tracker Green. Scale bars: 5 μm.

19. Confocal imaging of endogenous NO.

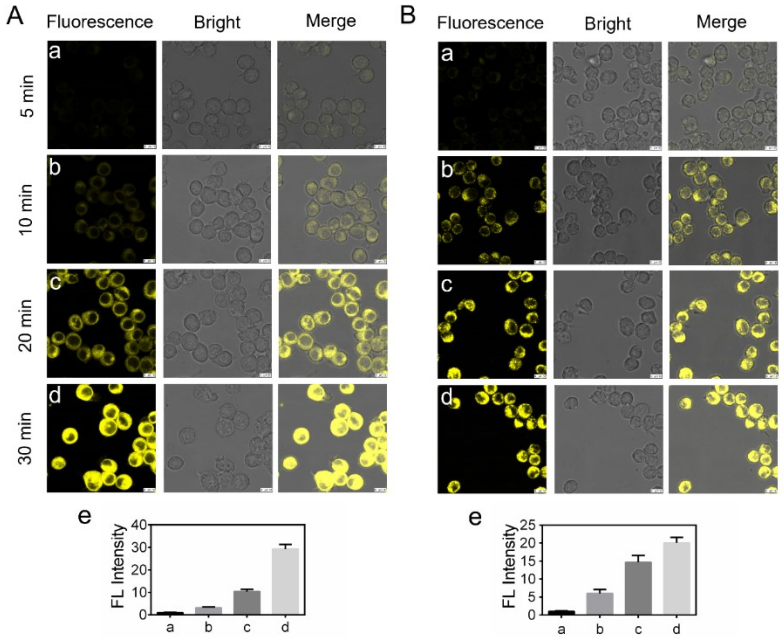


Figure S15. Confocal microscopy images of NO-rich RAW 264.7 cells incubated with (A) ER-BOD-1 or (B) Lyso-BOD-1 (5 μM) for (a) 5, (b) 10, (c) 20, and (d) 30 min. (e) Relative fluorescence intensities quantified from the corresponding images in (a-d). Scale bars: 10 μm.

20. Activator and inhibitor assays.

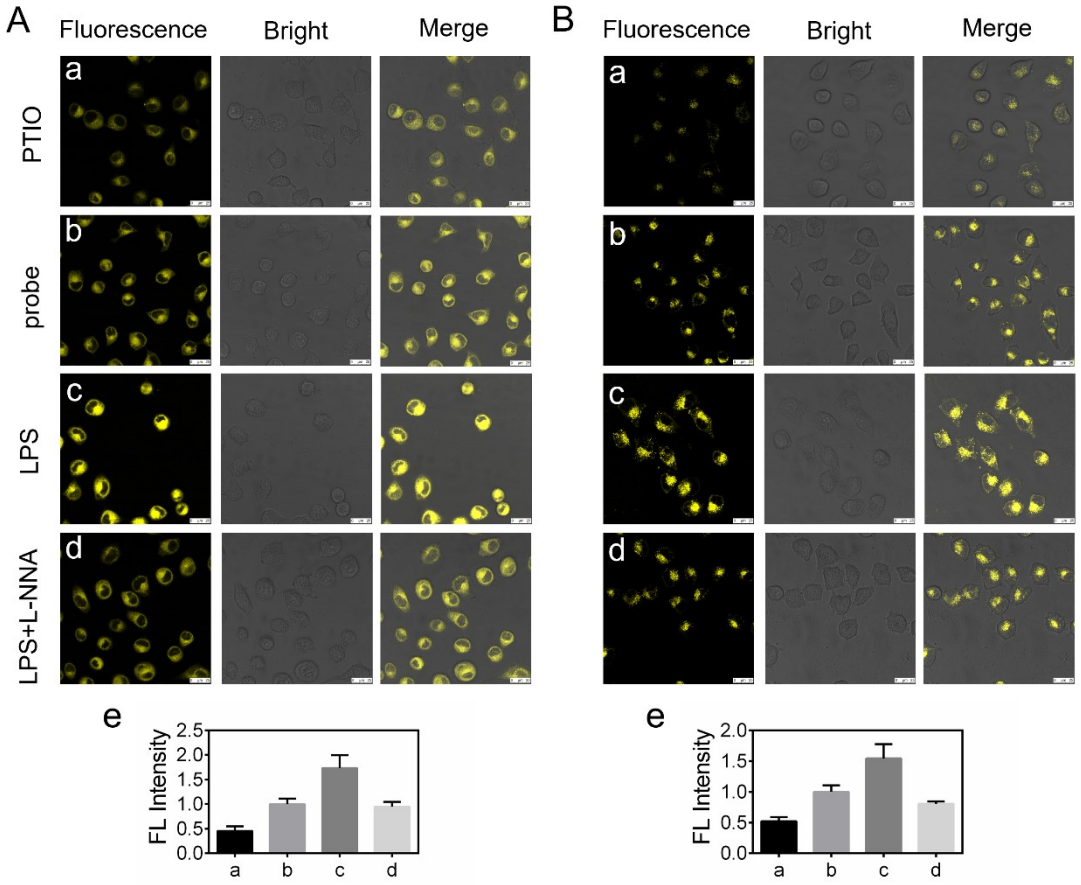
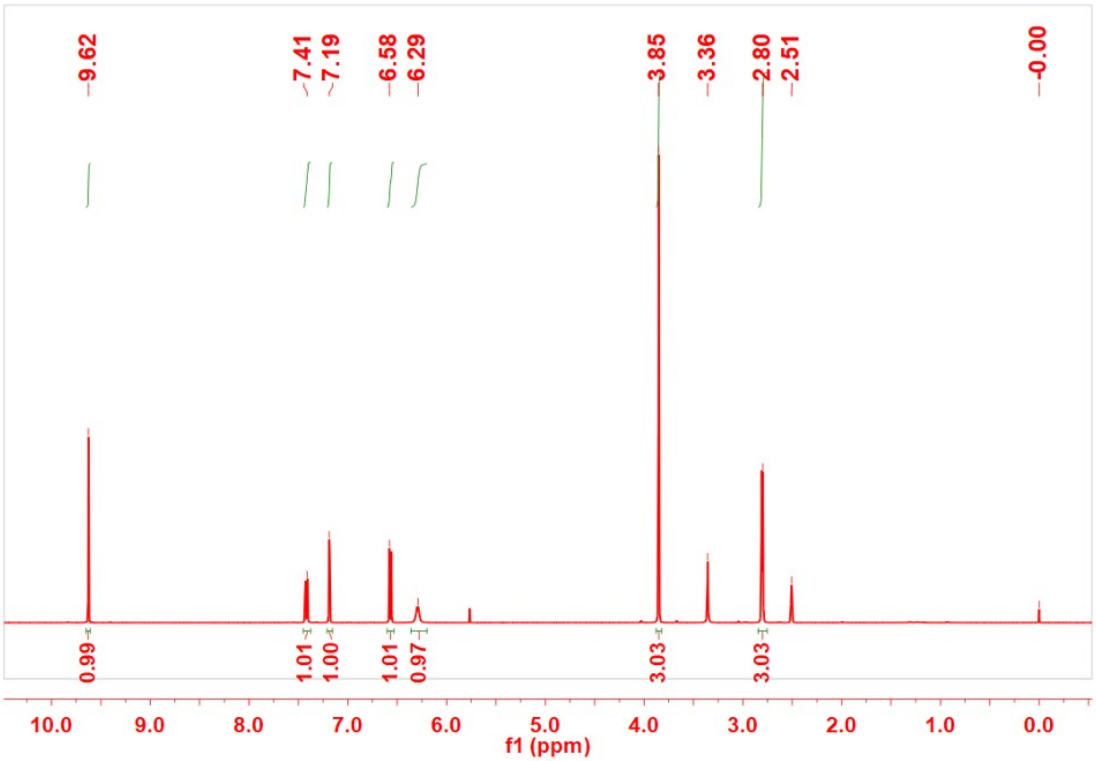
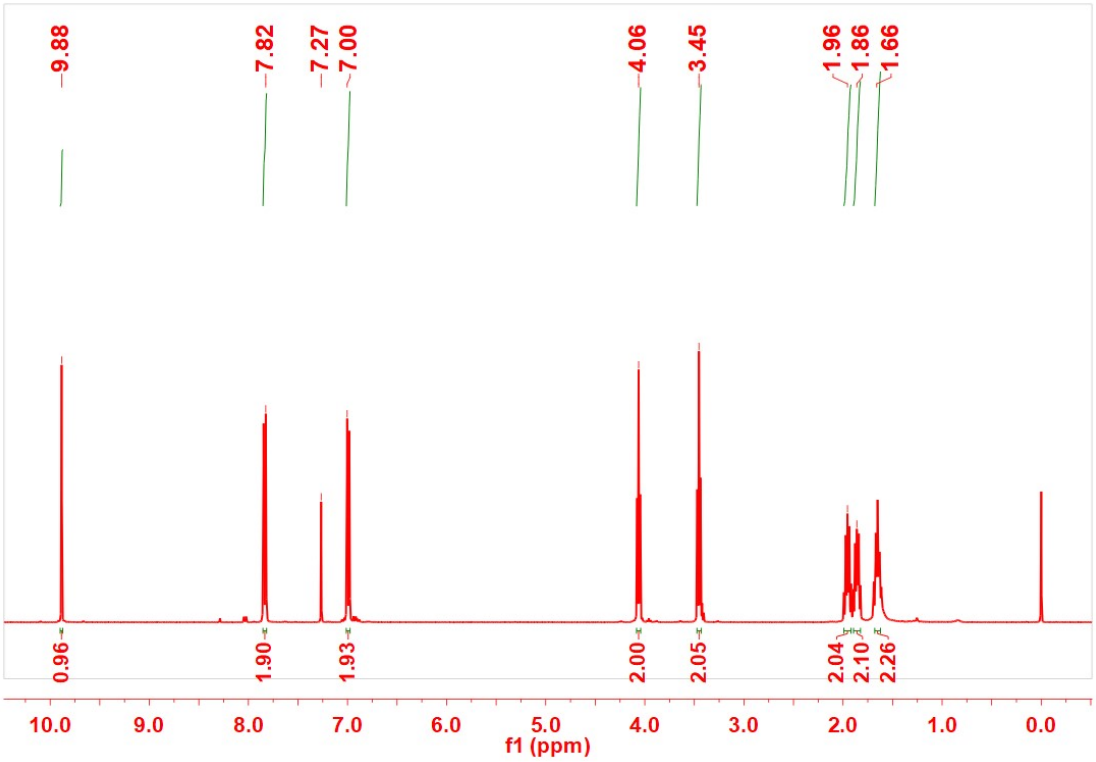


Figure S16. Cells pretreated with (a) PTIO (0.5 mM) for 30 min, (c) LPS (20 μg/mL) for 12 h, (d) LPS (20 μg/mL) and L-NNA (10 μM) for 12 h, then loaded with (A) ER-BOD-1 or (B) Lyso-BOD-1 (5 μM) for 30 min. (b) Cells loaded with ER-BOD-1 or Lyso-BOD-1 (5 μM) only for 30 min. (e) Relative fluorescence intensities quantified from the corresponding images in (a-d). Scale bars: 25 μm.

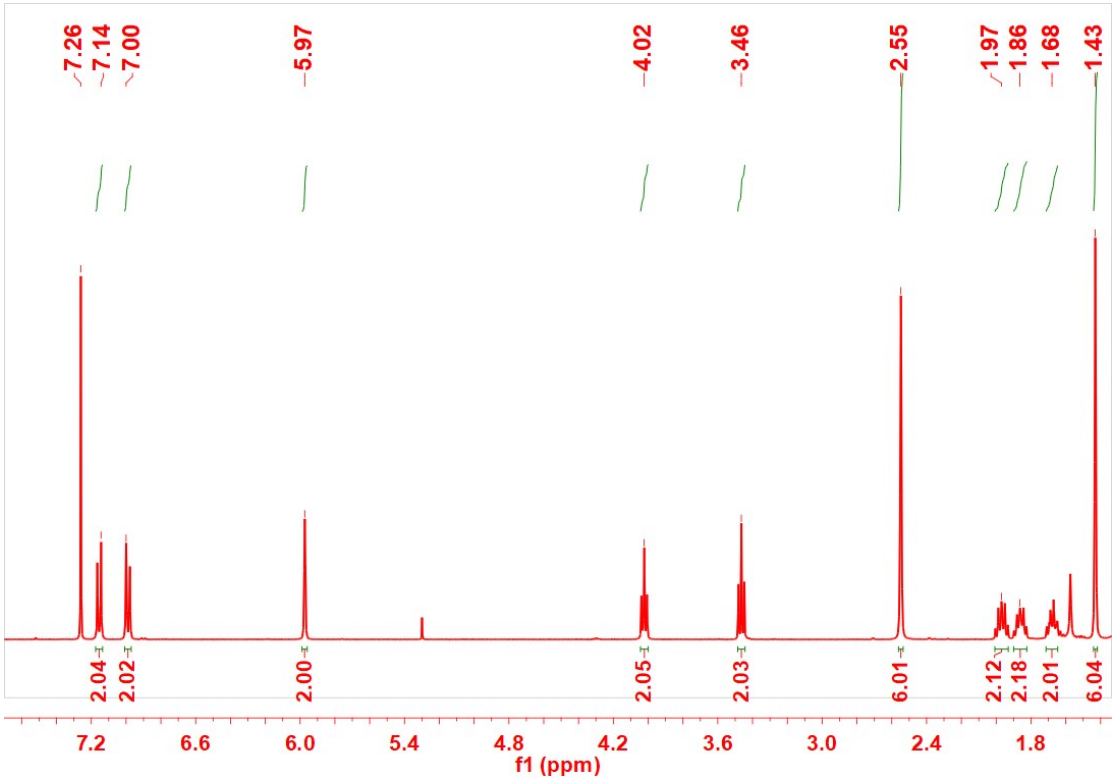
21. NMR and HRMS.



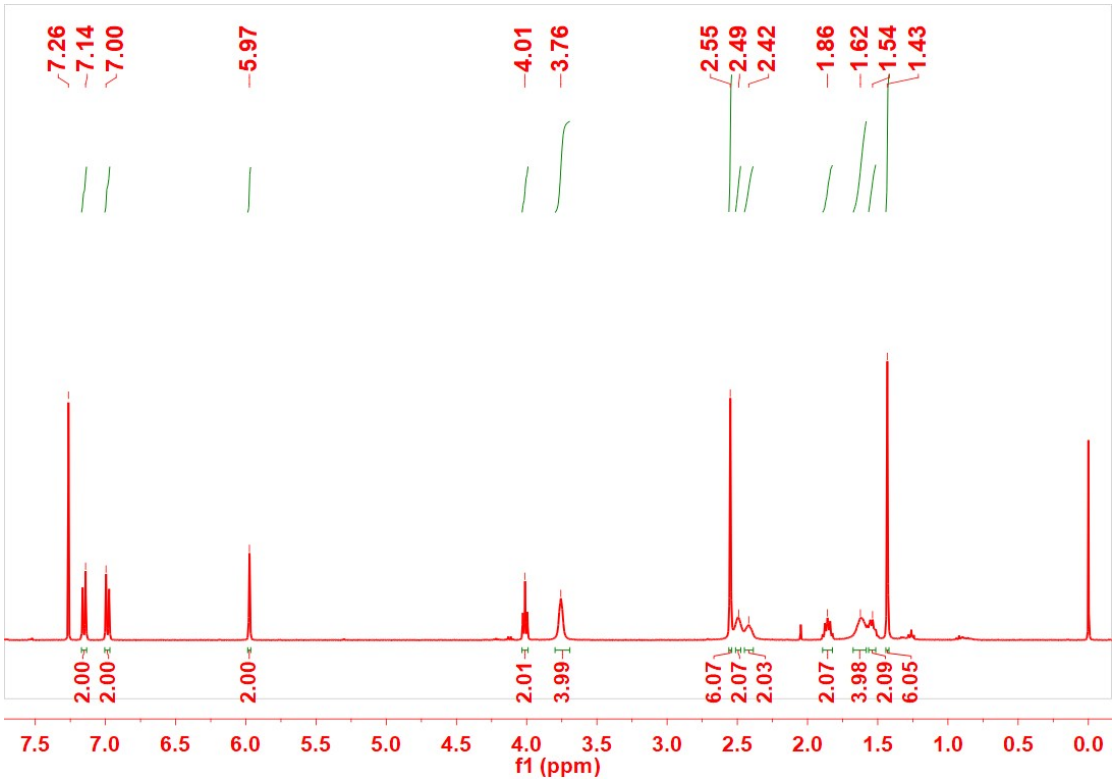
^{1}H NMR spectrum for compound 2.



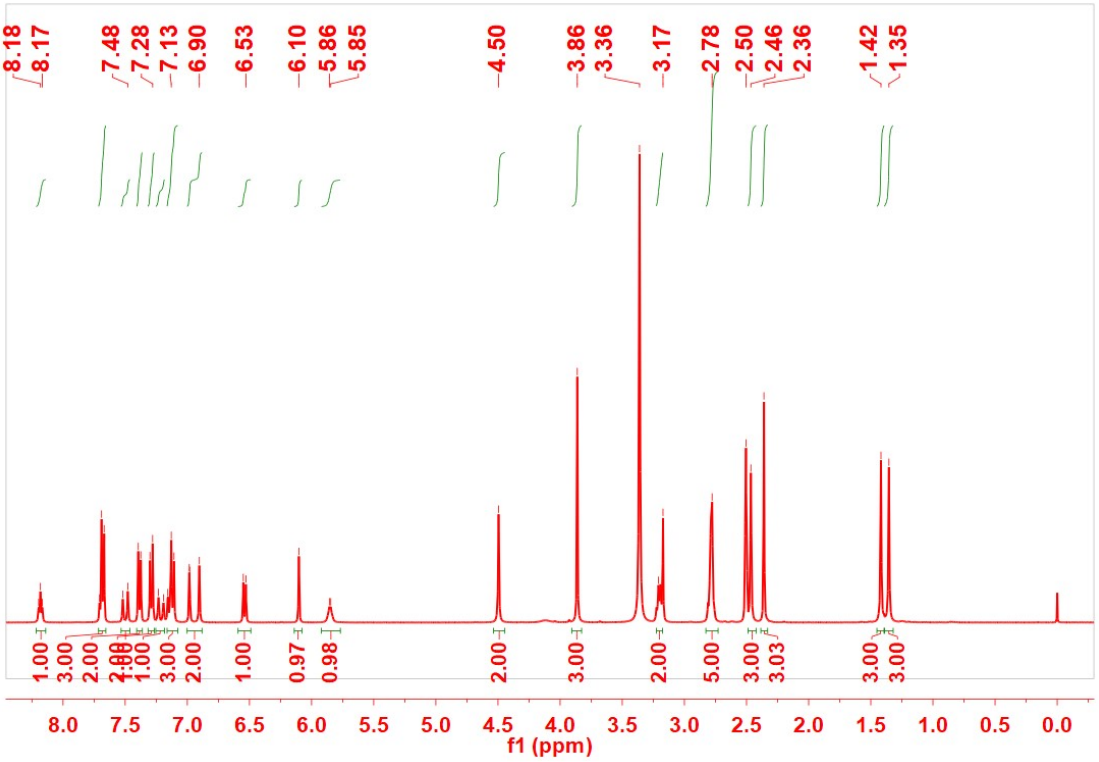
^{1}H NMR spectrum for compound 4.



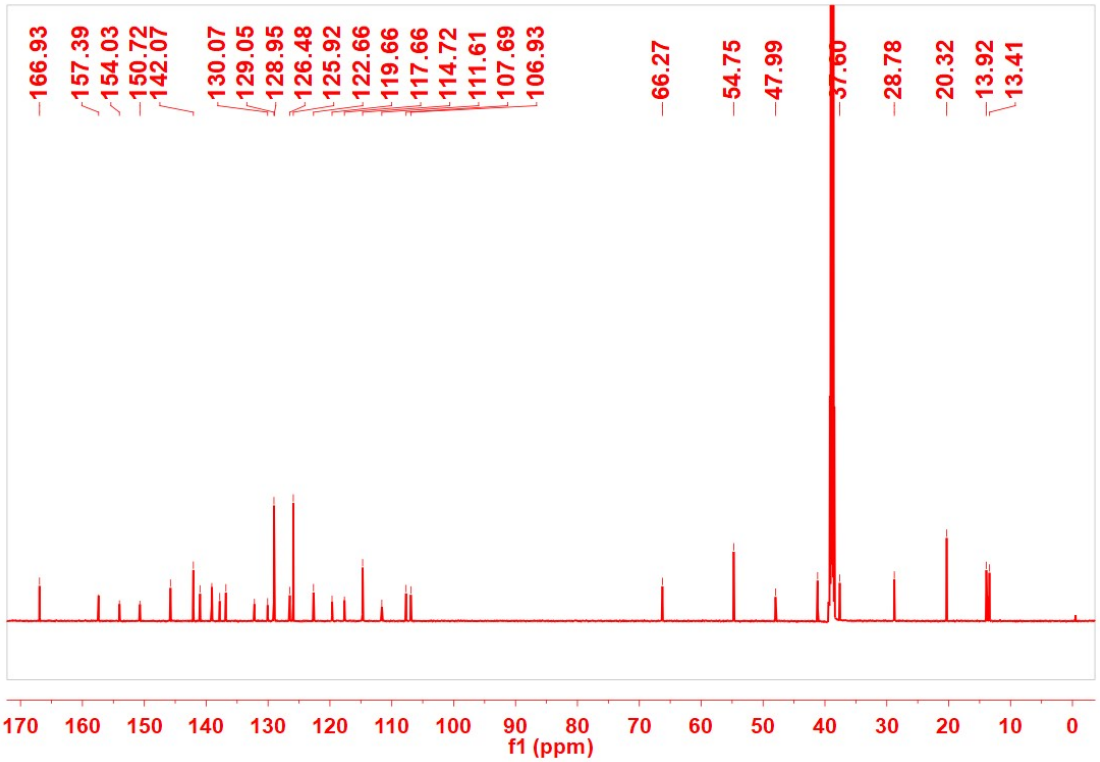
^{1}H NMR spectrum for compound 5.



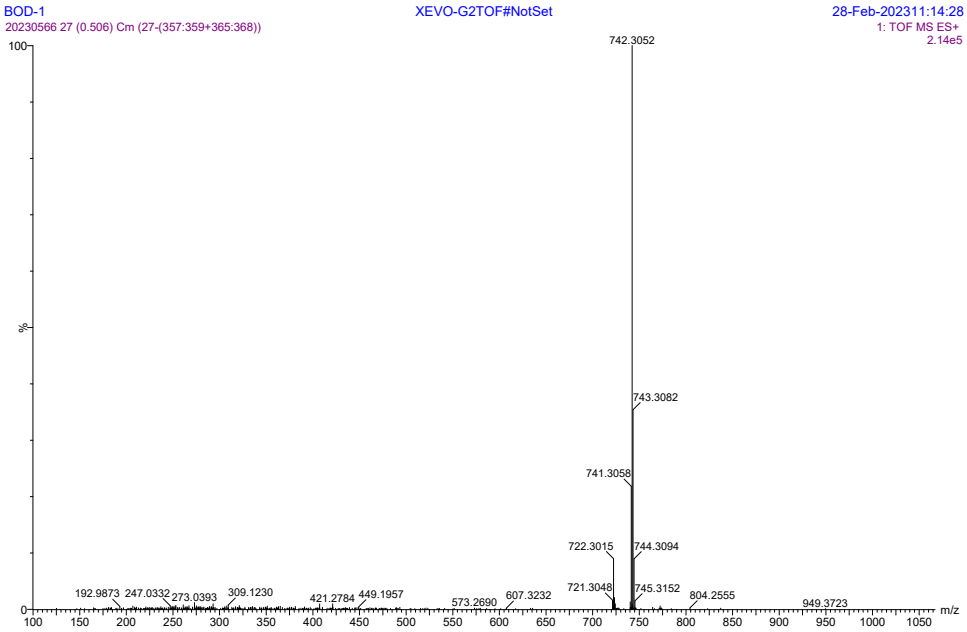
^{1}H NMR spectrum for compound Lyso-BOD.



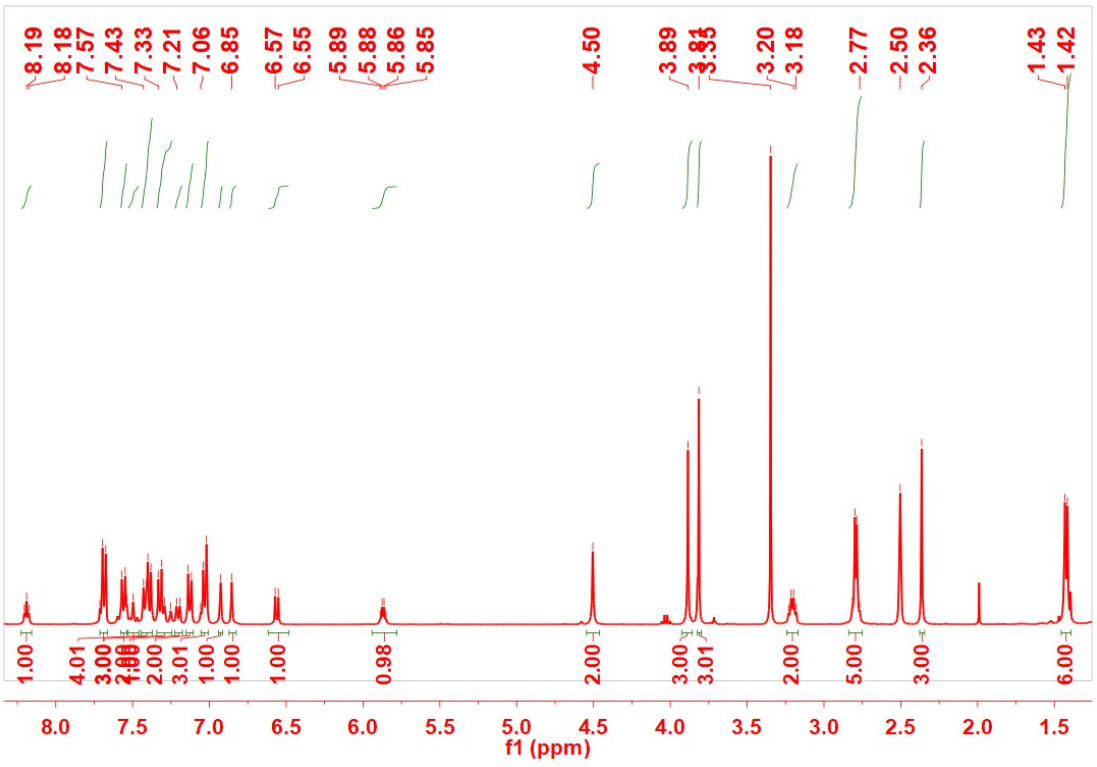
^{1}H NMR spectrum for compound ER-BOD-1.



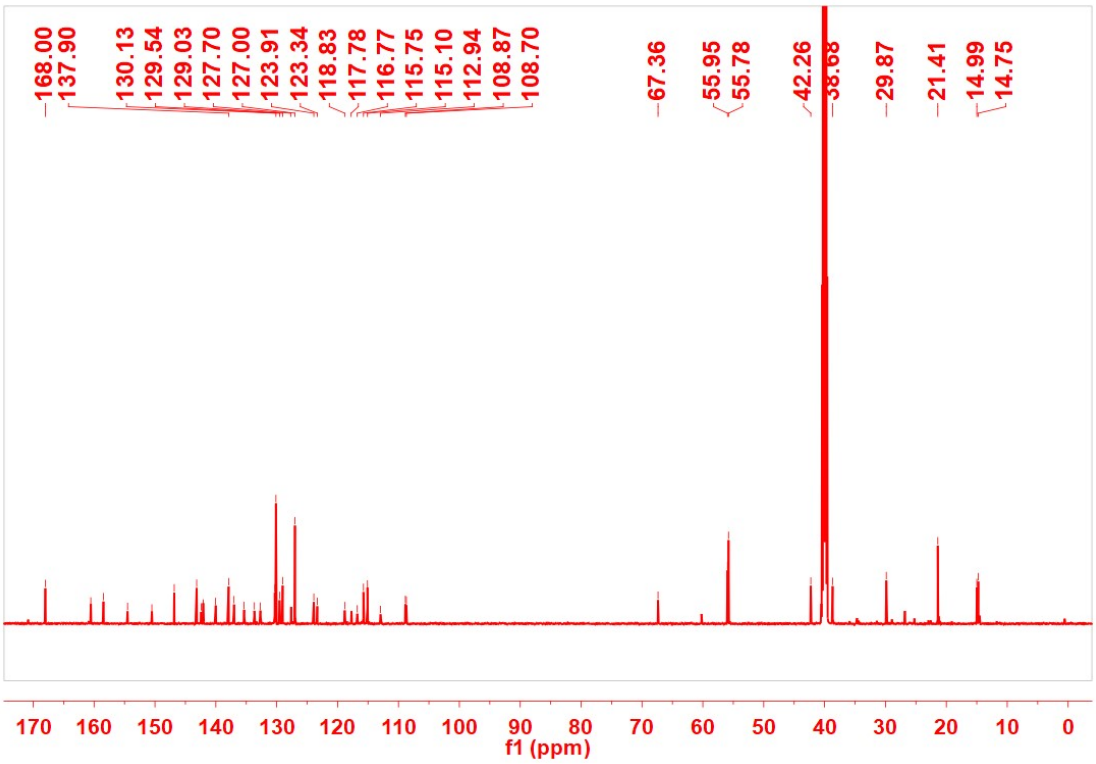
^{13}C NMR spectrum for compound ER-BOD-1.



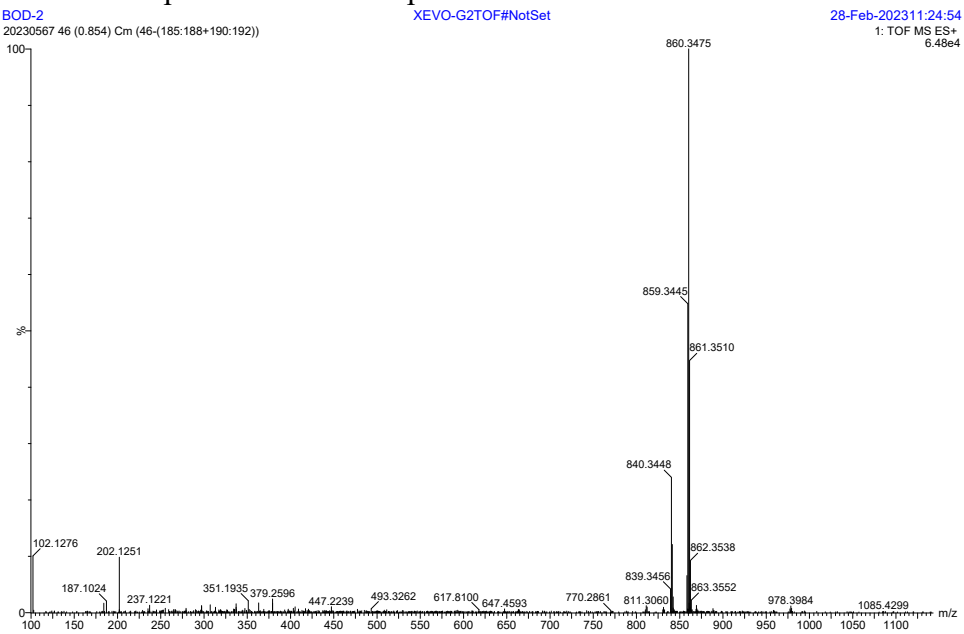
HRMS spectrum for compound ER-BOD-1.



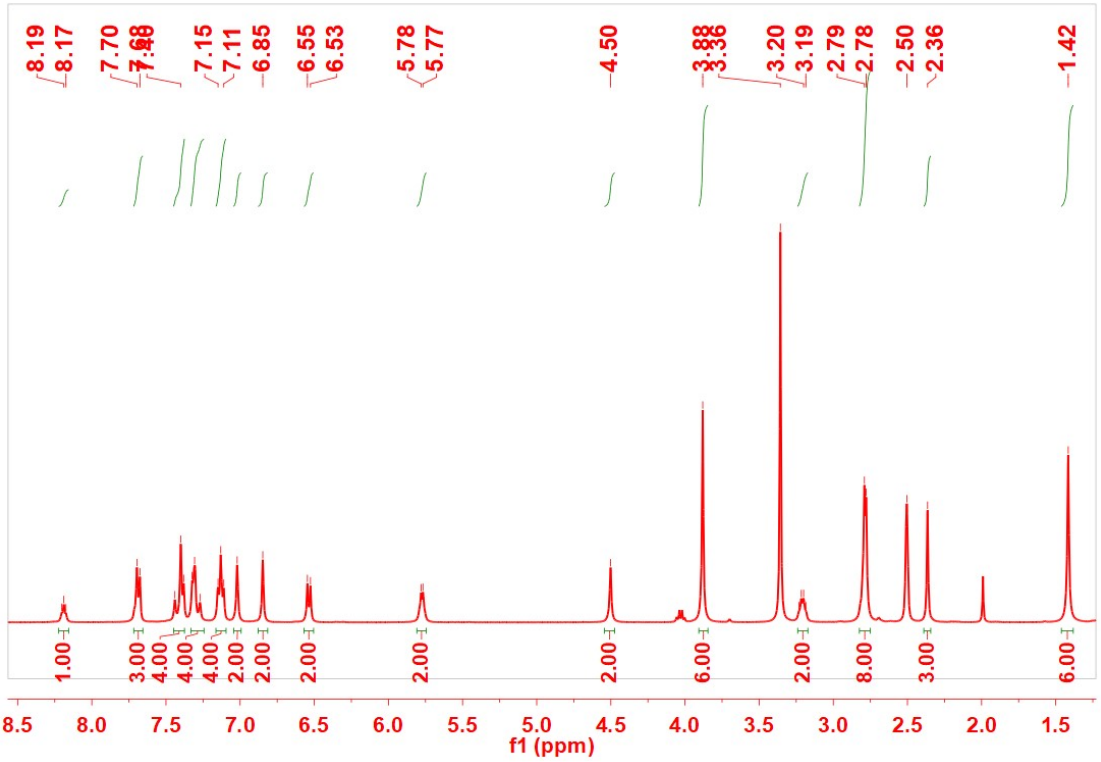
^{1}H NMR spectrum for compound ER-BOD-2.



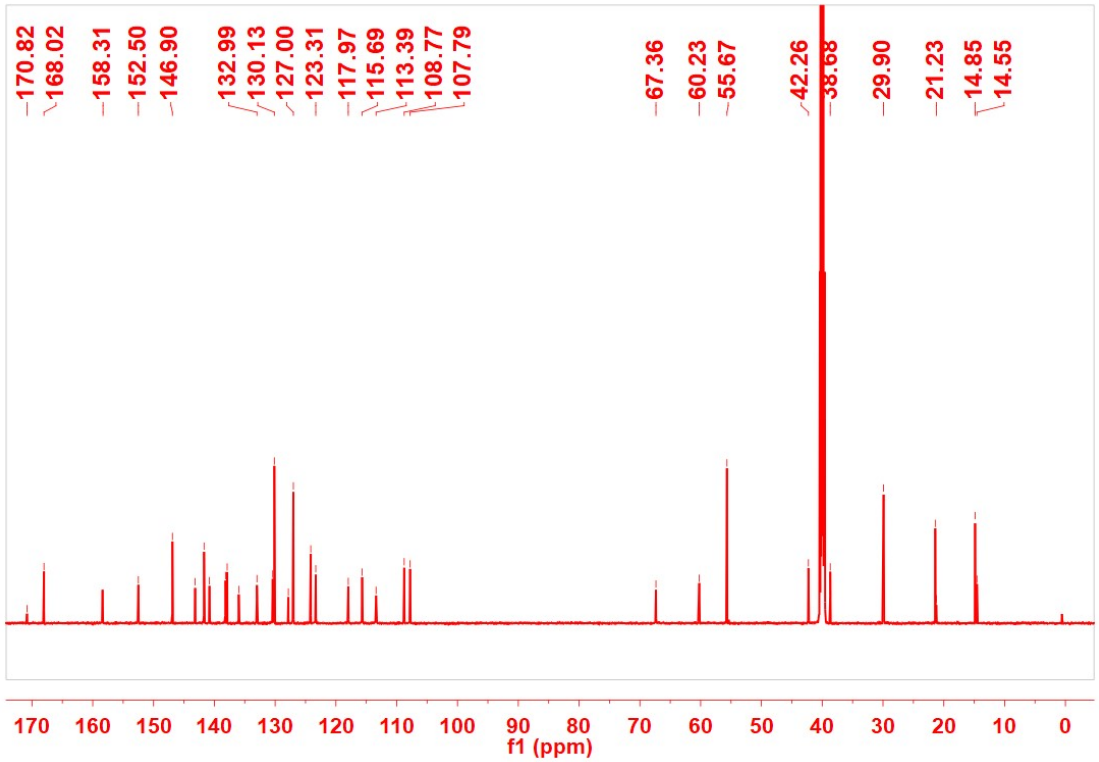
^{13}C NMR spectrum for compound ER-BOD-2.



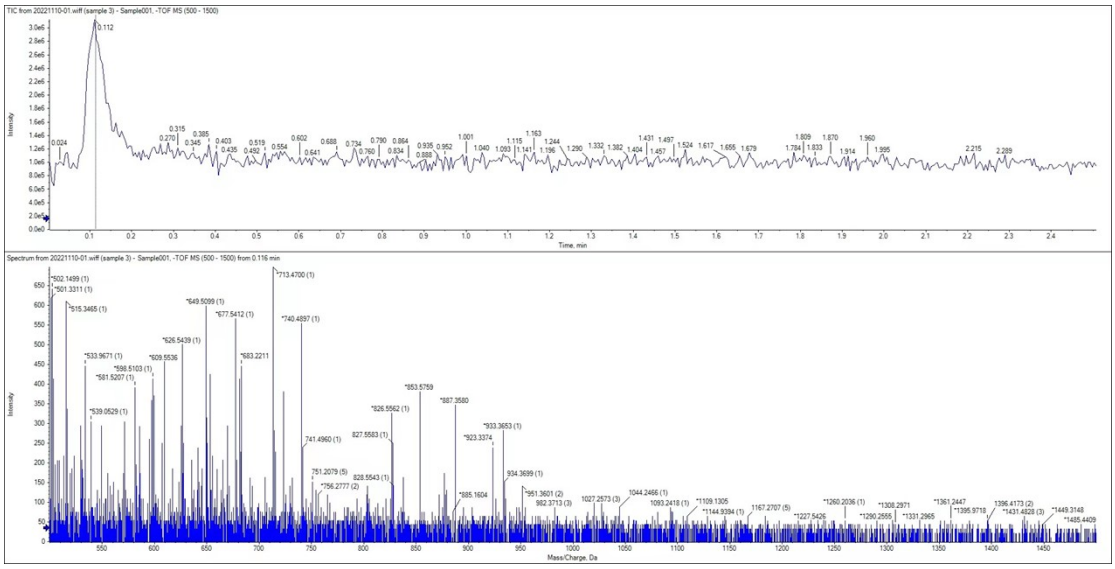
HRMS spectrum for compound ER-BOD-2.



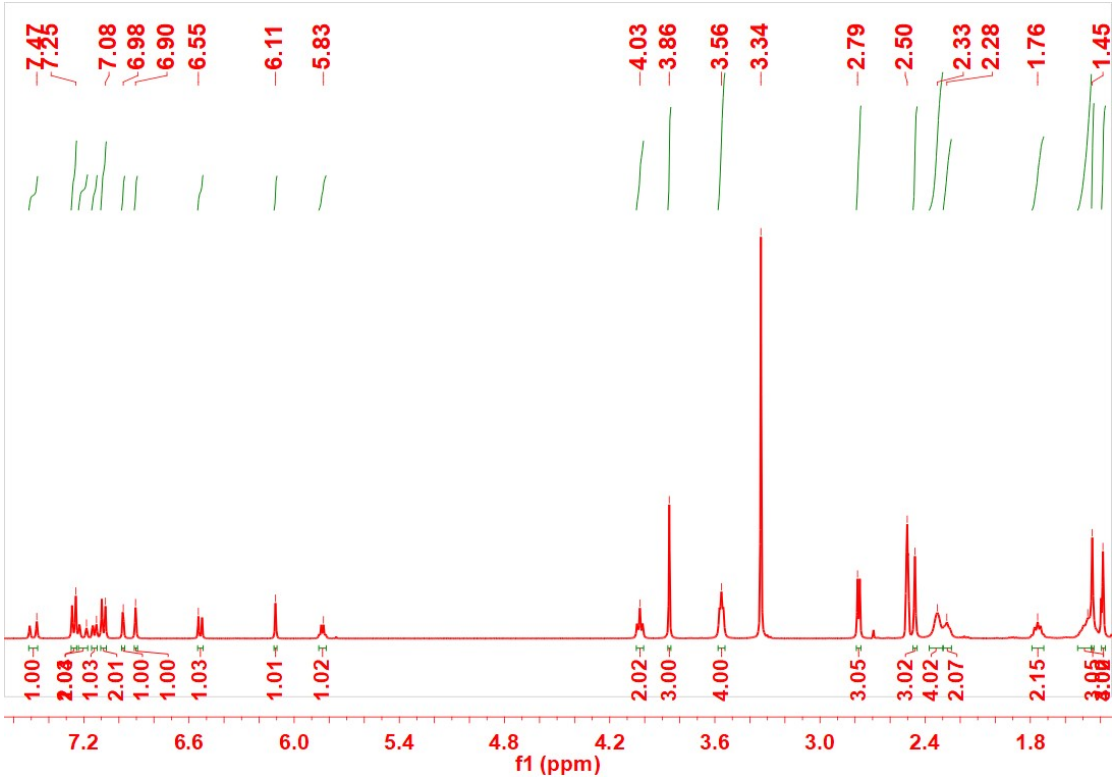
^{1}H NMR spectrum for compound ER-BOD-3.



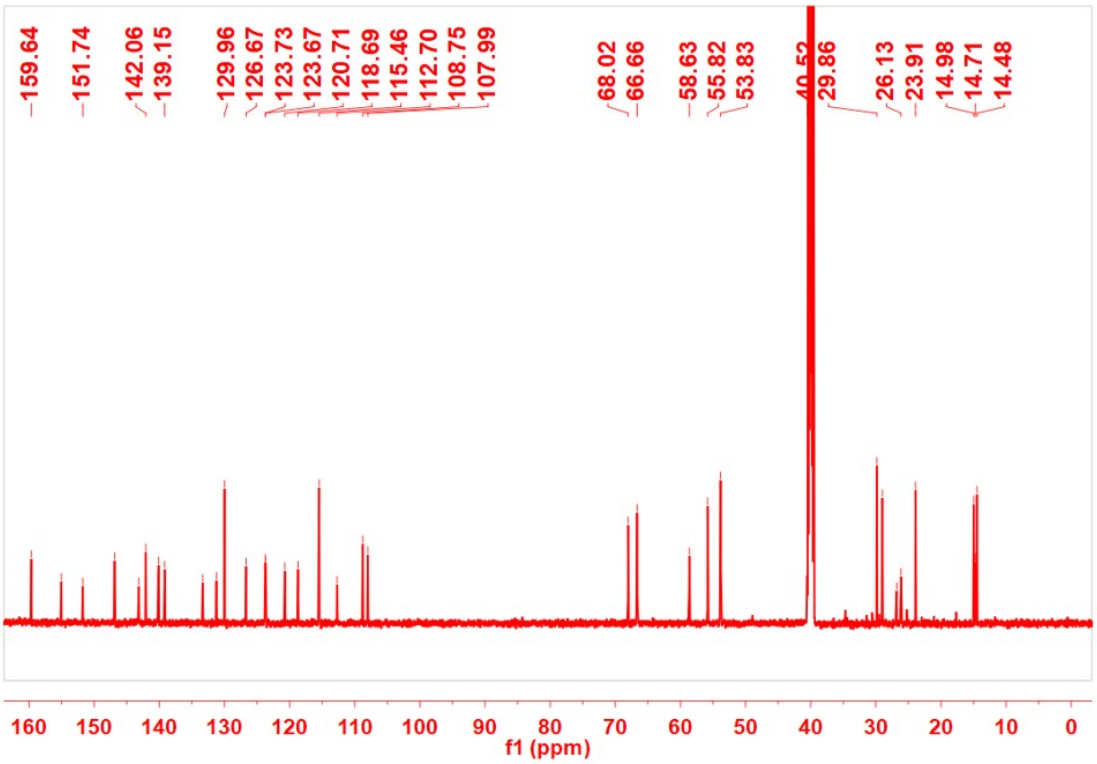
^{13}C NMR spectrum for compound ER-BOD-3.



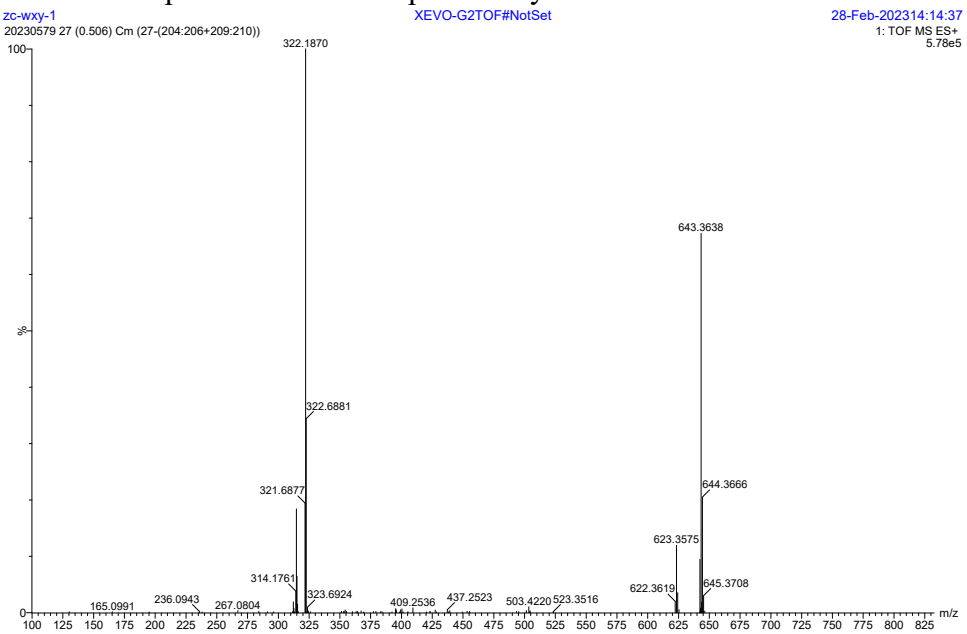
HRMS spectrum for compound ER-BOD-3.



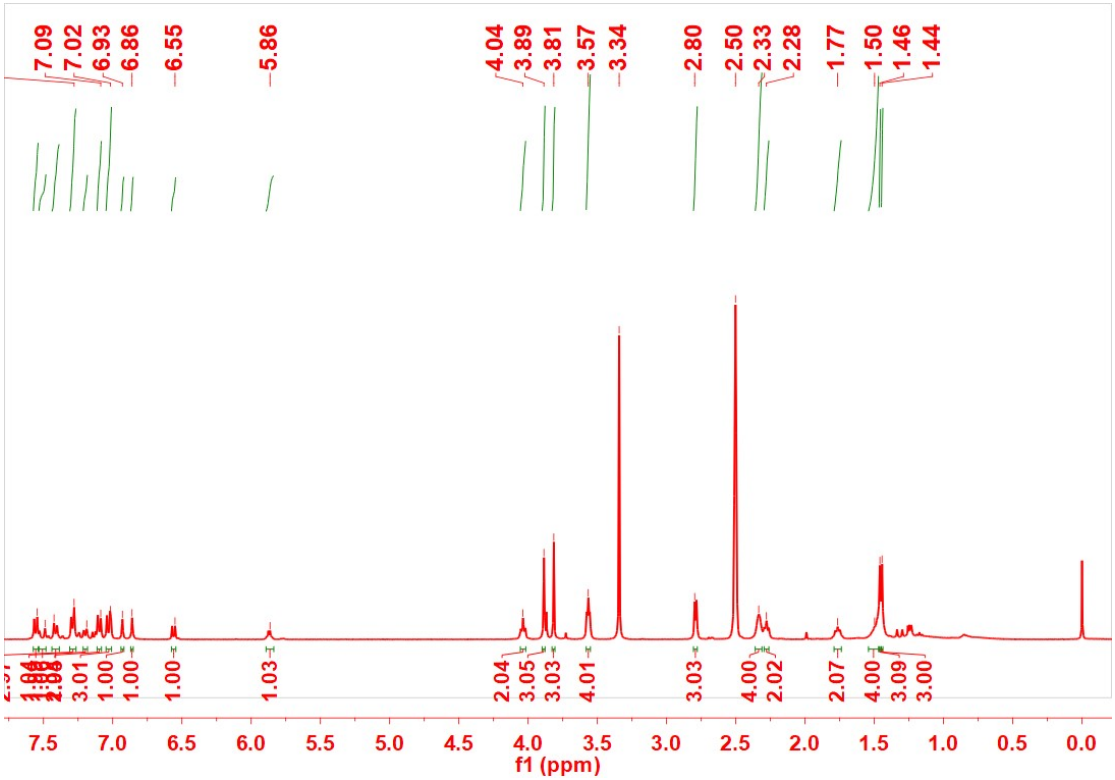
^{1}H NMR spectrum for compound Lyso-BOD-1.



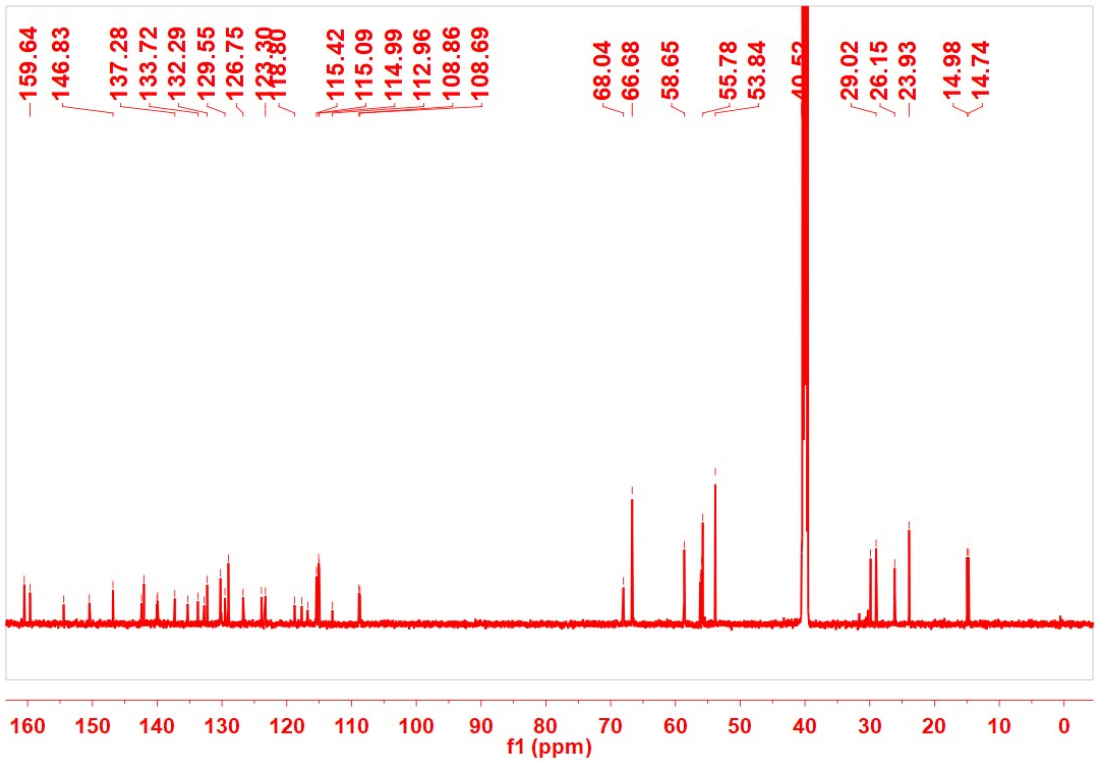
^{13}C NMR spectrum for compound Lyso-BOD-1.



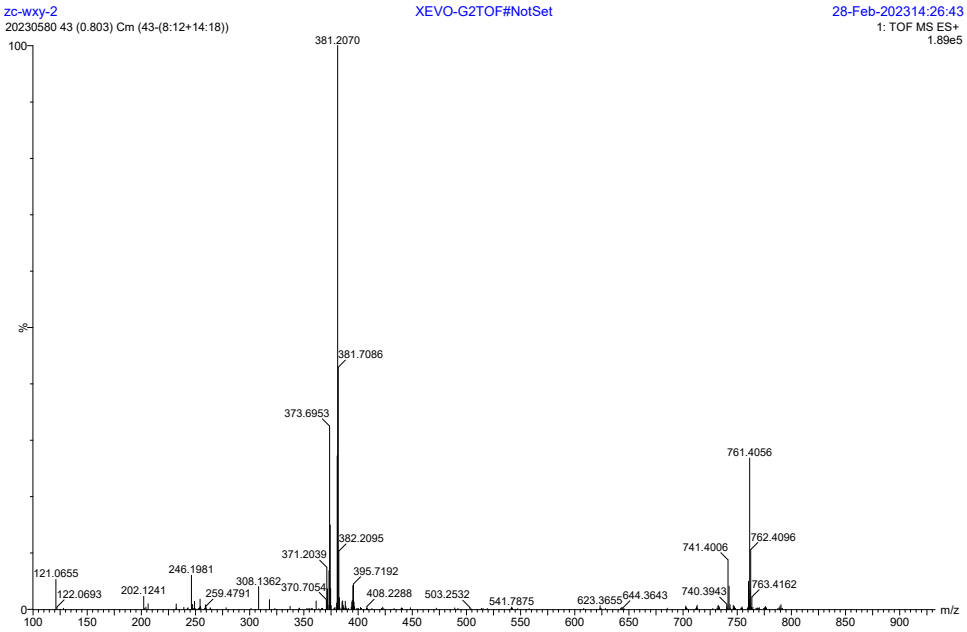
HRMS spectrum for compound Lyso-BOD-1.



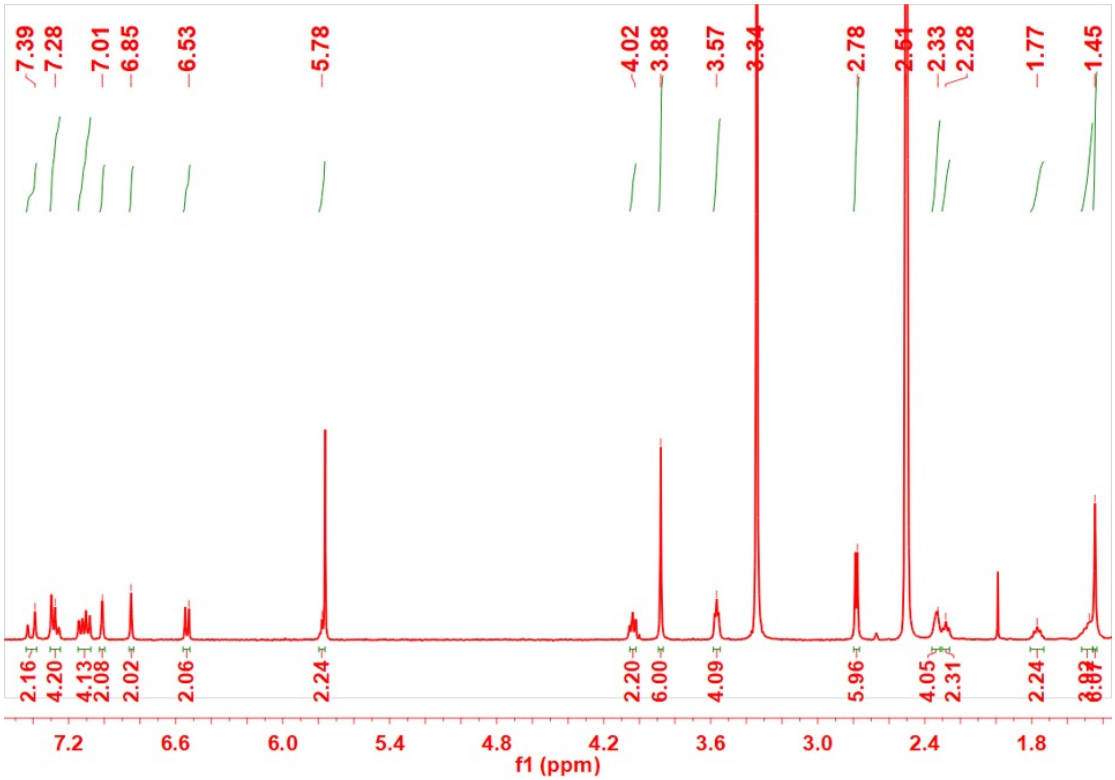
^{1}H NMR spectrum for compound Lyso-BOD-2.



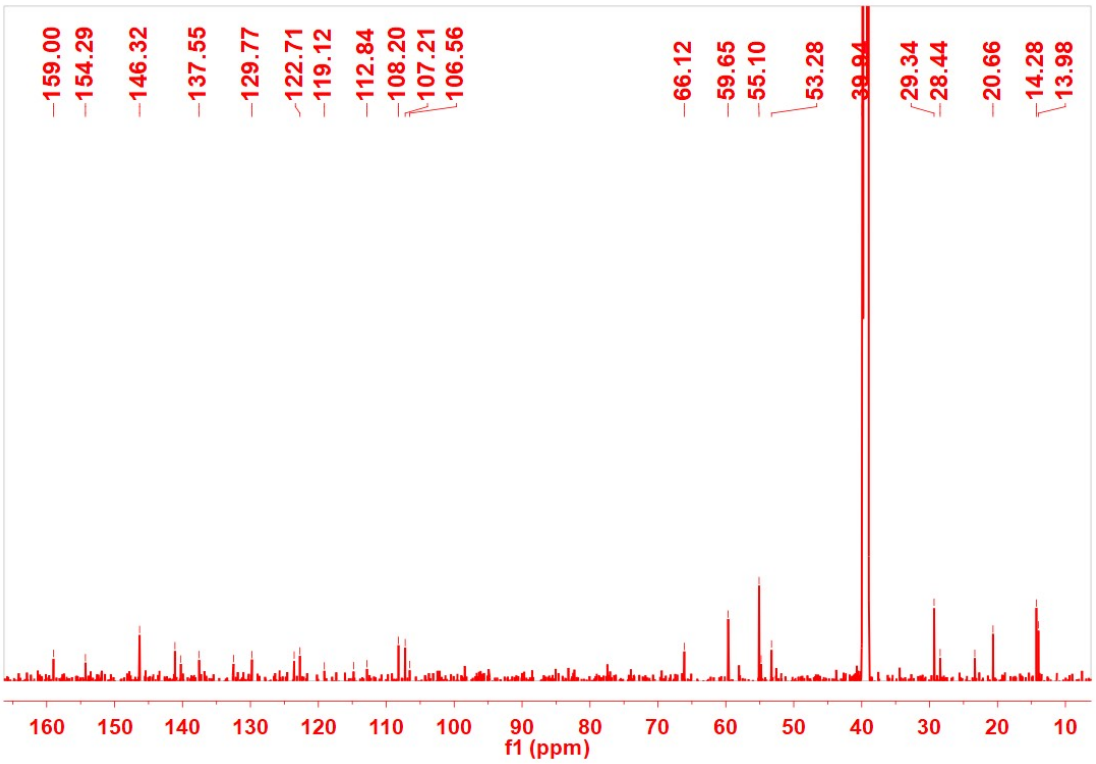
^{13}C NMR spectrum for compound Lyso-BOD-2.



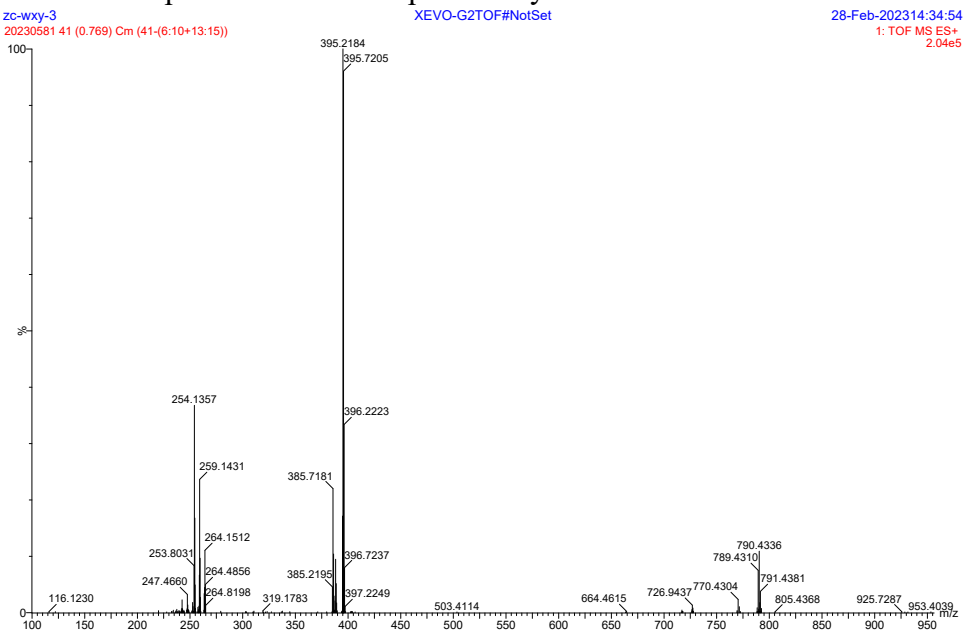
HRMS spectrum for compound Lyso-BOD-2.



^{1}H NMR spectrum for compound Lyso-BOD-3.



^{13}C NMR spectrum for compound Lyso-BOD-3.



HRMS spectrum for compound Lyso-BOD-3.

22. References.

1 Chen, R. Wang, R. Sun, J. X. Dong, C. Dong, L. Sun, X. Gu and C. Zhao, *Org. Biomol. Chem.*, 2023, **21**, 5919.