Supporting information

Self-Reporting Heavy Atom-Free Photodynamic Therapy Agents

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1. General Methods

All reagents and solvents were purchased from commercial sources and used without further purification. Column chromatography was carried out using silica stationary phase (230–400 mesh, SiliCycle Inc., Canada). Analytical thin layer chromatography was performed on 0.25 mm thick precoated silica gel plates (60F254, Merck, Germany). Compounds were visualized under UV light. All ^{1}H NMR spectra were recorded on a Varian Inova instrument (400 MHz) at Selçuk University, Konya. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (*J*) are reported in hertz (Hz). Standard abbreviations indicating multiplicities are given: br = broad, d = doublet, m = multiplet, s = singlet, t = triplet. High-resolution mass spectrometry was carried out using Agilent 6530 Accurate-Mass Q-TOF LC/MS of the Eastern Anatolia Advanced Technology Research and Application Centre (DAYTAM, Erzurum, Turkey). For PDT, LED from Bright LED Electronics Corp. and model BL-BG43V4V and BL-BJG344V-1-AA-AV with peak absorption values at 505 and 637 nm respectively was used as a light source. For cell culture experiments MCF7 human breast adenocarcinoma cell line (ATCC) were used. Cells were visualised with Zeiss Fluorescence Microscopy.

2. Additional Figures and

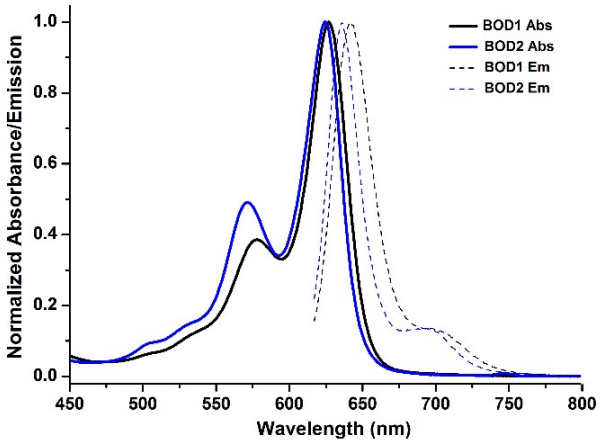


Figure S1. Normalized absorbance (solid) and fluorescence (dashed) spectra of BOD1 (black) and BOD2 (blue) in H_2O/acetonitrile (1:99; v/v).

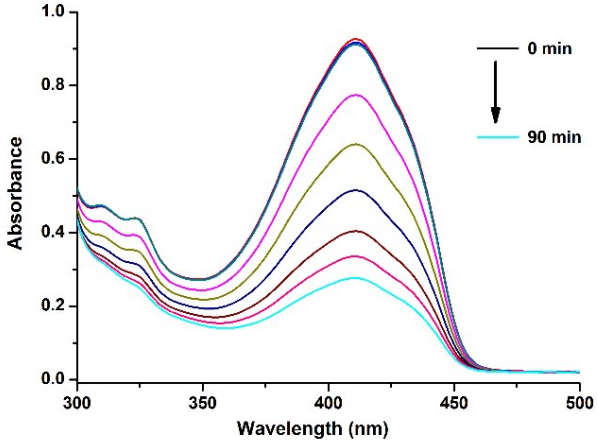


Figure S2. Decrease in the absorbance of DPBF (50 μM) in the presence of **BOD1** (2 μM) in %1H_2O in acetonitrile. Sample is kept in dark in the first 30 minutes, then irradiated with 637 nm LED light.

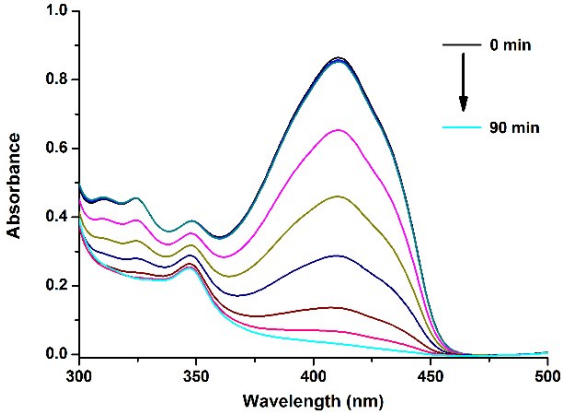


Figure S3. Decrease in the absorbance of DPBF (50 μM) in the presence of **BOD2** (2 μM) in %1H_2O in acetonitrile. Sample is kept in dark in the first 30 minutes, then irradiated with 637 nm LED light.

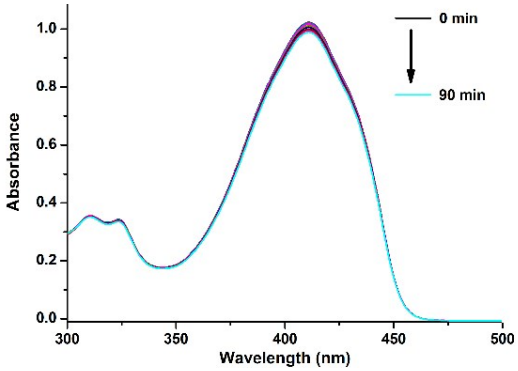


Figure S4. Decrease in the absorbance of DPBF alone (50 μM) in %1H_2O in acetonitrile. Sample is kept in dark in the first 30 minutes, then irradiated with 637 nm LED light.

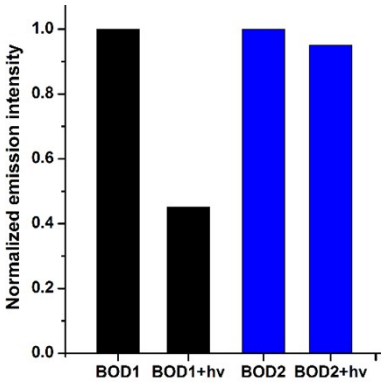
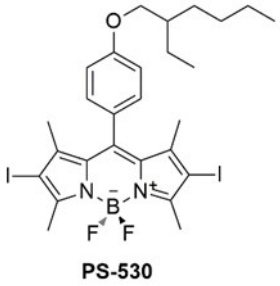


Figure S5. Normalized peak emission intensity of **BOD1** and **BOD2** at in the presence and absence of 637 nm light. Light is introduced by an LED source for 120 min from a distance of 5 cm.



PS-530

Figure S6. Structure of photosensitizer PS-530.

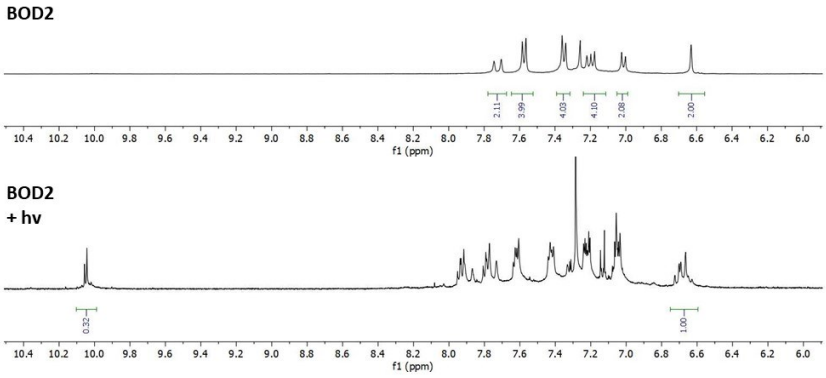


Figure S7. Change in ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of 10 mg **BOD2** upon 3h irradiation with 505 nm LED light from a distance of 5 cm in the presence of 1mg **PS-530**. New signal corresponding formyl proton appears at 10.02 ppm and other peaks split into multiple peaks indicating the presence of **BOD2**, **BOD2** with single conversion to aldehyde and **BOD2** with 2 formyl groups. Comparison of the peak intensities of newly generated aldehyde and pyrrolic protons of BODIPY around 6.60-6.70 ppm indicates 32% conversion of the photosensitizer upon irradiation.

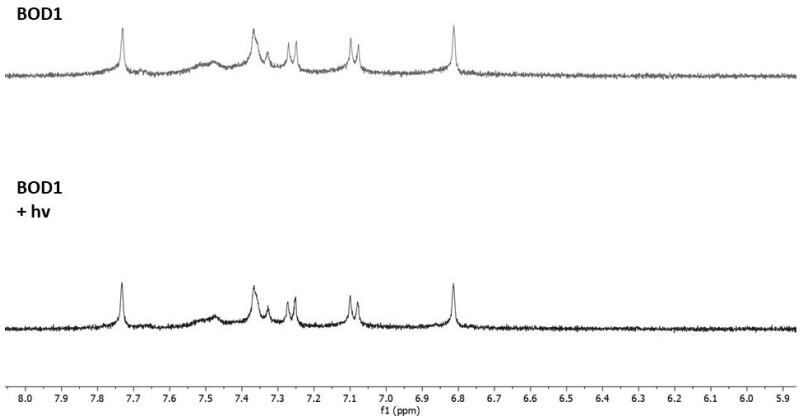


Figure S8. Change in ^{1}H NMR spectrum (*d*-DMSO, 400 MHz) of 5 mg **BOD1** upon 3h irradiation with 505 nm LED light from a distance of 5 cm. No change in the peak positions/intensities are detected.

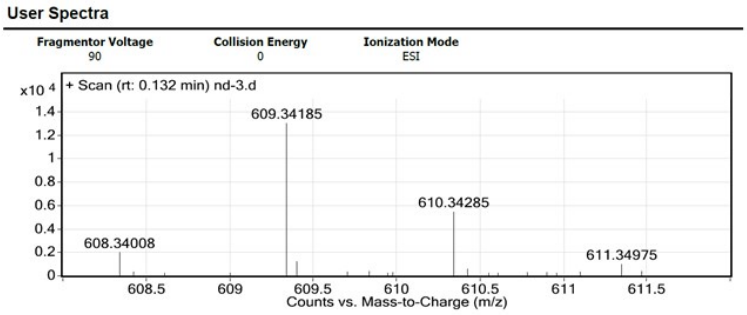


Figure S9. High Resolution ESI-MS Spectra of **BOD1** after 3h irradiation with 505 nm LED light from a distance of 5 cm. Values are 609.3325 for theoretical m/z of $(M+H)^+$ and 609.34185 for experimental (Δ: 13.13 ppm).

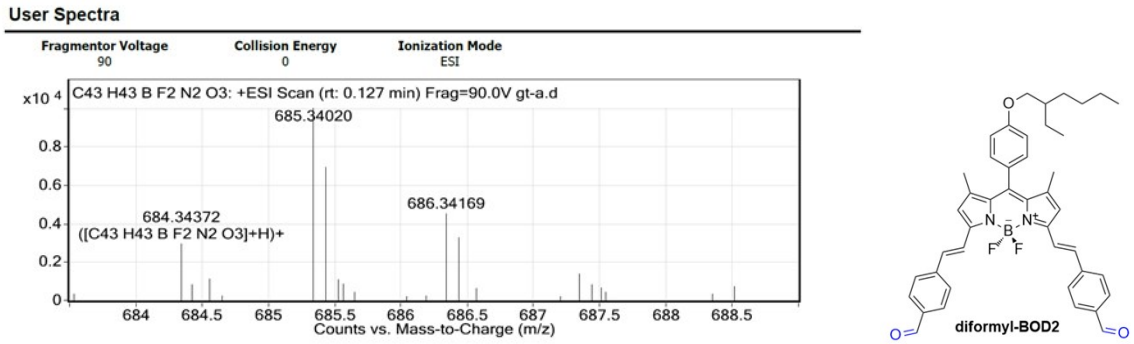


Figure S10. High Resolution ESI-MS Spectra of **BOD2** after 3h irradiation with 505 nm LED light from a distance of 5 cm. Values for diformyl-**BOD2** (shown on the right) are 685.3413 for theoretical m/z of $(M+H)^+$ and 685.34020 for experimental (Δ: 1.61 ppm).

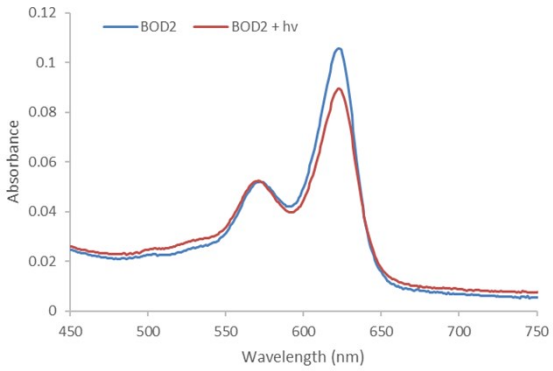


Figure S11. Change in UV-Vis Absorbance spectrum of **BOD2** after 2h irradiation with 637 nm light. Small decrease in absorption is contributed to formyl-**BOD2** formation.

^{1}H NMR spectra, High Resolution Mass spectra and UV-Vis spectra of **BOD1** and **BOD2** (Figures S7-S11) support the stability of these compounds after irradiation with light. NMR and HRMS analysis indicates that **BOD2** is converted into formyl-**BOD2** as described in the main text whereas **BOD1** does not change chemically.

3. Synthesis and Other Experimental Procedures:

Singlet Oxygen Generation Experiments

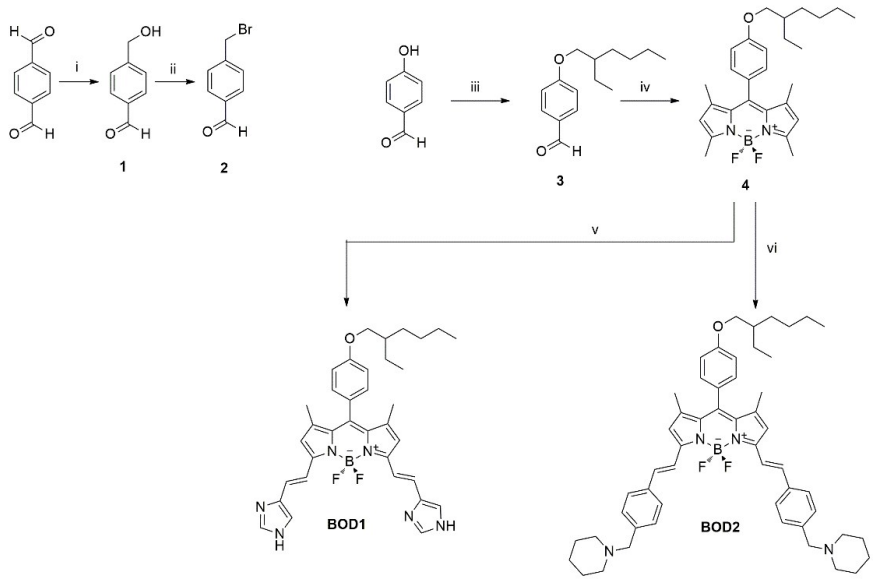
Singlet oxygen generation is followed by spectral analysis of the singlet oxygen trap 1,3-diphenyl isobenzofurane. Absorption of this compound at 411 nm decreases upon reaction with $^1O_2$. 50 μM of DPBF is prepared in H_2O/acetonitrile solvent mixture (1:99 v/v). Samples are purged with air for 5 min. UV-Vis Absorbance spectra were recorded using Agilent Cary Eclipse 60 Spectrophotometer in 10 min intervals either with or without photosensitizers (2μM). Samples are kept under dark for the first 30 min and then irradiated with LED lamp of given wavelength (505 or 637 nm) from a 5 cm distance. Fluorescence spectra are recorded with the use of 1μM and 5 μM **BOD1** and **BOD2** respectively using Agilent Cary Eclipse Fluorescence Spectrophotometer. PS-530 photosensitizer (5 μM) is used to clearly observe $^1O_2$ dependent fluorescence change of photosensitizers when irradiated at a different wavelength (505 nm) where PSs are not absorbing.

Change in the absorption of DPBF (50 μM) at 411 nm upon $^1O_2$ generation by pre-irradiated photosensitizers **BOD1** (2μM) and **BOD2** (2 μM) in 1% H_2O in acetonitrile is measured with the same procedure described above. Samples are purged with air for 5 min, irradiated with 637 nm light for 1.5h from a 5 cm distance prior to experiment. Then, samples are kept in dark for 30 min and later irradiated with 637 nm LED light. Absorption spectra of DPBF is recorded in every 10 min throughout the experiment.

Cell Culture Experiments

Cell culture experiments are done using MCF7 breast cancer cells. Cells are seeded in 96-well plate and incubated for 24h at 37^{o}C under 5% CO_2. **BOD1** and **BOD2** stock solutions are prepared in dimethyl sulfoxide, then diluted with culture medium and then introduced to cells with the final concentration of 5μM each. Cells are further incubated at 37^{o}C either under dark for 24h or under 637 nm irradiation for initial 4h and later for 20h under dark. Cells are washed with PBS, dissociated from the surface with the use of Tripsin-EDTA solution. After washing with RPMI-1640 medium cells are counted by Tryphan Blue staining on Thoma chamber and IC50 values are calculated. Cells were visualised with Zeiss Fluorescence Microscopy. MTT assay with MCF7 cells was performed in 96-well microplate. The cells viability was estimated by measuring absorbance at 570 nm using a Quant ELISA plate reader (Bio-tek Instruments, USA). Images were captured using an inverted light microscope.

Synthesis



Scheme S1. Synthesis of **BOD1** and **BOD2**. Reaction conditions: i) $NaBH_4$, THF, EtOH; ii) NBS, PPh_3, DCM; iii) K_2CO_3, 2-ethylhexyl bromide, DMF; iv) a. 2,4-dimethyl pyrrole, N_2, TFA, DCM; b) p-chloranil; c) Et_3N, $BF_3.OEt_2$; v) 4-imidazole carboxaldehyde, piperidine, acetic acid, benzene; vi) compound 2, piperidine, acetic acid, benzene.

Synthesis Compound 1: Terephthalaldehyde (10 g, 75 mmol) was dissolved in 125 ml ethanol and 175 ml tetrahydrofuran. The reaction mixture is cooled to 5^{o}C. $NaBH_4$ (0.85 g, 22.5 mmol) was added to reaction mixture in small aliquots over 30 minutes. Reaction is followed by TLC and after 1.5h, quenched with 2M HCl solution. Solvents are evaporated under reduced vacuo and crude sample was extracted with ethyl acetate. White solid product is obtained after crystallization with 81 % yield.

^{1}H NMR (*CDCl*$_3$, 400 MHz, δ ppm) 9.92 (s, ArC*H*O, 1H), 7.80 (d, J=8.1 Hz, Ar*H*; 2H), 7.46 (d, J=7.9 Hz, Ar*H*; 2H), 4.72 (s, *O*CH_2; 2H), 4.61 (b, OH; 1H).

^{13}C NMR (*CDCl*$_3$, 400 MHz, δ ppm) 192.60, 148.09, 136.13, 130.39, 127.01, 65.09.

High Resolution ESI-MS values are 137.0597 for theoretical m/z of $(M+H)^+$ and 137.0586 for experimental (Δ: 8.03 ppm)

Synthesis Compound 2: Compound 1 (1 g, 7.35 mmol) was dissolved in 50 ml dichloromethane (DCM). N-Bromosuccinimide (1.36 g, 7.64 mmol) was added to the reaction mixture. Then, triphenylphosphine (3.85g, 14.68 mmol) was added in small aliquots over 30 min. The reaction is stirred at room temperature and followed by TLC. When all the starting material was consumed, crude product is extracted with dichloromethane. Organic layer was collected, dried over sodium sulphate and solvent is removed under vacuo. Crude product is purified using silica column chromatography using petroleum ether and ethyl acetate as mobile phase (25:1; v/v). White solid product is obtained with 65% yield.

^{1}H NMR (*$CDCl_3$*, 400 MHz, δ ppm) 10.00 (s, ArC*H*O, 1H), 7.85 (d, J=8.2 Hz, Ar*H*; 2H), 7.55 (d, J=8.1 Hz, Ar*H*; 2H), 4.51 (s, *Br*CH_2; 2H).

^{13}C NMR (*$CDCl_3$*, 400 MHz, δ ppm) 208.41, 191.94, 144.70, 136.42, 130.39, 32.20.

Synthesis Compound 3: 4-hydroxybenzaldehyde (4 g, 33 mmol) was dissolved in 30 ml dimethylformamide (DMF). K_2CO_3 (13.6 g, 99 mmol) and K_2CO_3 and 2-ethylhexylbromide (6.7 ml, 37 mmol) were added to reaction mixture and stirred for 16h at room temperature. Solid precipitates were filtered out and filtrate was evaporated under vacuo. Filtrate was dissolved in dichloromethane and extracted with water. Organic layer was collected, dried over sodium sulphate and solvent is removed under vacuo. Crude product is purified using silica column chromatography using dichloromethane as mobile phase. Colourless liquid product is obtained with 98% yield.

^{1}H NMR (*$CDCl_3$*, 400 MHz, δ ppm) 9.90 (s, ArC*H*O, 1H), 7.84 (d, J = 8.50 Hz , Ar*H*; 2H), 7.03 (d, J = 8.70 Hz, Ar*H*; 2H), 3.95 (d, J = 5.70 Hz, *O*CH_2; 2H), 1.77 (m, C*H*; 1H), 1.50-1.30 (m, C*H_2*; 8H), 0.95 (m, CH_2C*H_3*; 6H).

^{13}C NMR (*$CDCl_3$*, 400 MHz, δ ppm) 190.96, 164.58, 132.08, 129.74, 114.82, 70.92, 39.32, 30.51, 29.12, 23.86, 23.10, 14.17, 11.76.

High Resolution ESI-MS values are 235.1692 for theoretical m/z of $(M+H)^+$ and 235.1693 for experimental (Δ: 0.42 ppm).

Synthesis of Compound 4. 300 ml dichloromethane was purged with N_2 for 20 minutes. Under inert athmosphere, compound **3** (10 mmol, 2.4 g) and 2,4 – dimethylpyyrole (22 mmol, 2.3 ml) were added.

Catalytic amount of trifluoroacetic acid (4-5 drops) were added, the color of the solution turned into brick-red. The solution was stirred at room temperature for 16h under N_2 athmosphere. p-chloranil (10 mmol, 2.5 g) was added to the solution and the reaction mixture was stirred for additional 3 hours at room temperature. Later, triethylamine (9 ml) and boron trifluoride-diethyl etherate (9 ml) were added sequentially. The reaction mixture was stirred for additional 3 hours at room temperature and was extracted with water and dichloromethane. Organic layer was collected, dried with sodium sulfate and dichloromethane was evaporated in vacuo. The crude product was purified by silica gel column chromotography using hexane and ethyl acetate mixture as mobile phase (4:1; v/v). Compound **4** was collected as orange oil with a yield of 35%.

^{1}H NMR (*$CDCl_3$*, 400 MHz, δ ppm) 7.15 (d, J = 8.69 Hz, Ar*H*; 2H), 7.04 (d, J = 8.72 Hz, Ar*H*; 2H), 6.02 (s, ArH, 2H), 3.94 (d, J = 5.89 Hz, OCH_2; 2H), 2.60 (s, ArCH_3; 6H), 1.81 (m, C*H*; 1H), 1.60-1.30 (m, ArCH_3 + CH_2; 14H), 0.97 (m, CH_2CH_3; 6H).

^{13}C NMR (*$CDCl_3$*, 400 MHz, δ ppm) 159.98, 155.18, 143.20, 142.07, 131.89, 129.10, 126.73, 121.06, 115.16, 70.85, 39.41, 30.54, 29.15, 23.87, 23.03, 14.60, 14.57, 14.10, 11.16.

High Resolution ESI-MS values are 453.2889 for theoretical m/z of $(M+H)^+$ and 453.29146 for experimental (Δ: 5.51 ppm).

Synthesis of BOD1. 160 mg 0.35 mmol compound 4 was dissolved in 10 ml of benzene. 2.5 mole equivalent of 4-imidazole carboxaldehyde (0.88 mmol, 85 mg), 250 µl piperidine and 250 µl acetic acid were added into the reaction mixture. Reaction was refluxed using Dean Stark apparatus until the color of the solution turned into blue-green colour. Then, reaction is followed by thin layer chromatography using methanol and dichloromehane mobile phase (10:90; v/v) until all compound 4 is consumed. Then the flask is cooled to room temperature, sample is extracted with dichloromethane. Organic layer was collected, dried with sodium sulfate and dichloromethane was evaporated in vacuo. The crude product was purified by silica gel column chromotography using methanol and dichloromethane mixture as mobile phase (10:90; v/v). **BOD1** was collected as blue-green solid with a yield of 11%.

^{1}H NMR (*d*-DMSO, 400 MHz, δ ppm) 7.73 (s, 2H, ArH), 7.48 (b, 2H, ArCH), 7.41-7.30 (m, ArH + ArCH, 4H), 7.26 (d, J = 8.6 Hz, 2H, ArH), 7.09 (d, J = 9.0 Hz, 2H, ArH), 6.83 (s, 2H, ArH), 5.44 (b,

2H, NH), 3.93 (d, J = 3.9 Hz, 2H, CH2), 1.69 (b, 1H, CH), 1.56-1.15 (m, ArCH3 + CH2, 14H), 0.88 (m, 12H, CH3).

^{13}C NMR and cannot be obtained because of poor solubility of the compound.

Synthesis of BOD2. 160 mg 0.35 mmol compound 4 was dissolved in 10 ml of benzene. 2.5 mole equivalent of compound 2 (0.88 mmol, 175 mg), 300 µl piperidine and 300 µl acetic acid were added into the reaction mixture. Reaction was refluxed using Dean Stark apparatus until the color of the solution turned into green colour. Then, reaction is followed by thin layer chromatography using methanol and dichloromehane mobile phase (10:90; v/v) until all compound 4 is consumed. Then the flask is cooled to room temperature, sample is extracted with dichloromethane. Organic layer was collected, dried with sodium sulfate and dichloromethane was evaporated in vacuo. The crude product was purified by silica gel column chromotography using methanol and dichloromethane mixture as mobile phase (10:90; v/v). **BOD2** was collected as green solid with a yield of 68%.

^{1}H NMR (*CDCl*$_3$, 400 MHz, δ ppm) 7.72 (d, *J* = 16.3 Hz, ArCH, 2H), 7.57 (d, *J* = 8.0 Hz, ArH, 4H), 7.35 (d, *J* = 8.0 Hz, ArH, 4H), 7.25 – 7.08 (m, ArH + ArCH, 4H), 7.01 (d, *J* = 8.6 Hz, ArH, 2H), 6.63 (s, ArH, 1H), 3.91 (d, *J* = 5.8 Hz, OCH2, 2H), 2.41 (b, NCH2, 8H), 1.77 (m, CH, 1H), 1.69 – 1.18 (m, ArCH3 + CH2, 26H), 1.07 – 0.83 (m, CH3, 6H).

^{13}C NMR (*CDCl*$_3$, 400 MHz, δ ppm) 160.25, 152.73, 142.40, 139.71, 139.42, 136.11, 135.66, 134.04, 129.89, 129.78, 127.57, 127.13, 119.24, 117.87, 115.34, 71.11, 63.72, 54.65, 39.65, 30.78, 29.92, 29.38, 26.15, 24.56, 23.27, 15.09, 14.32, 11.39.

High Resolution ESI-MS values are 823.5292 for theoretical m/z of $(M+H)^+$ and 823.5379 for experimental (Δ: 10.56 ppm).

4. NMR and HRMS Spectra

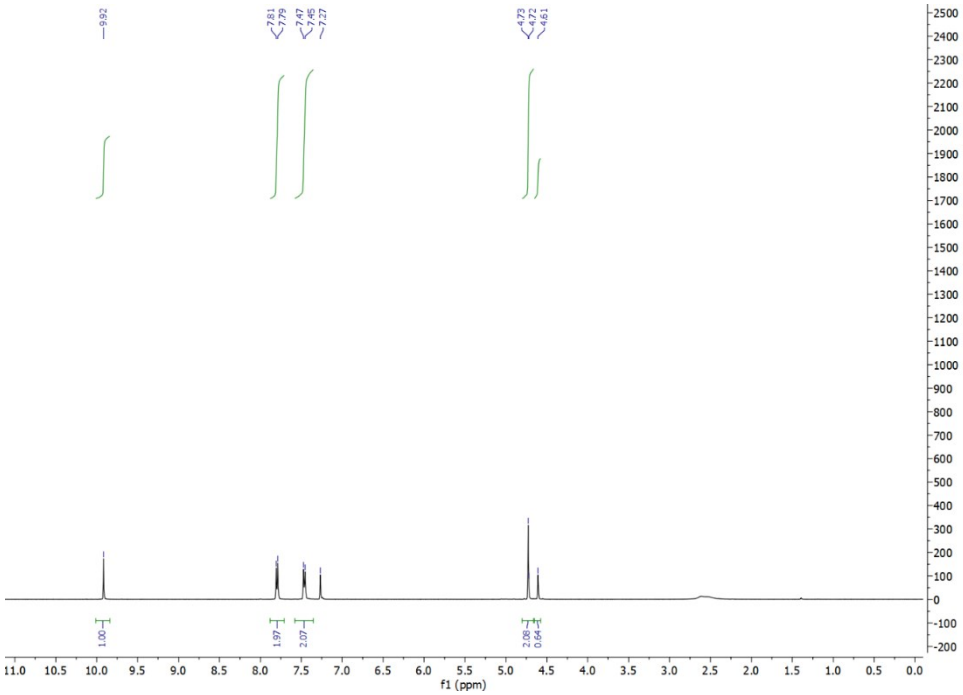


Figure S9. ^{1}H NMR Spectra of compound **1** (400 MHz, $CDCl_3$).

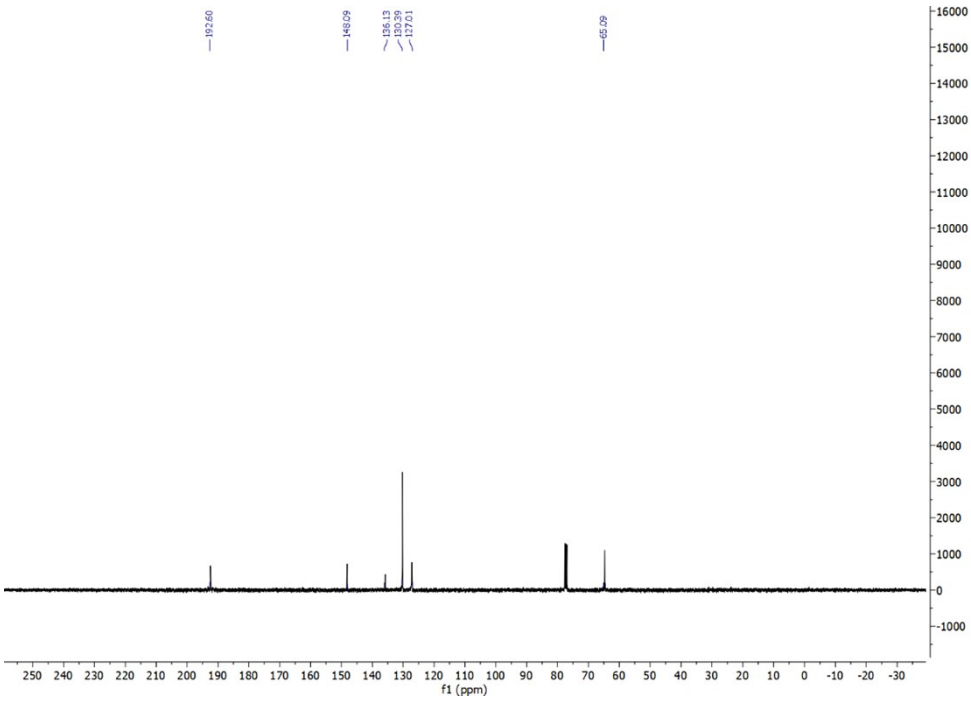


Figure S10. ^{13}C NMR Spectra of compound **1** (400 MHz, $CDCl_3$).

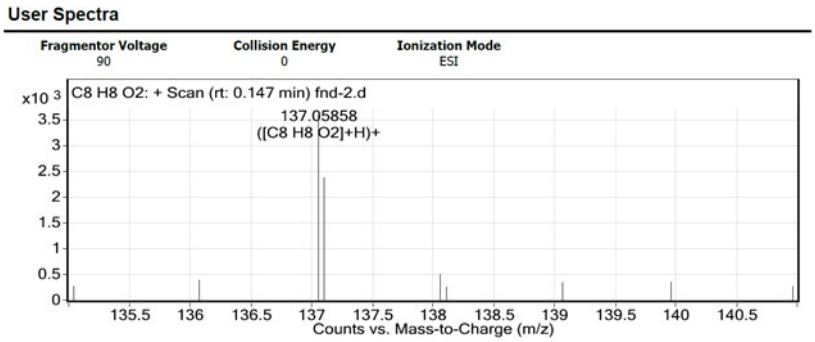


Figure 11. High Resolution ESI-MS of compound **1**.

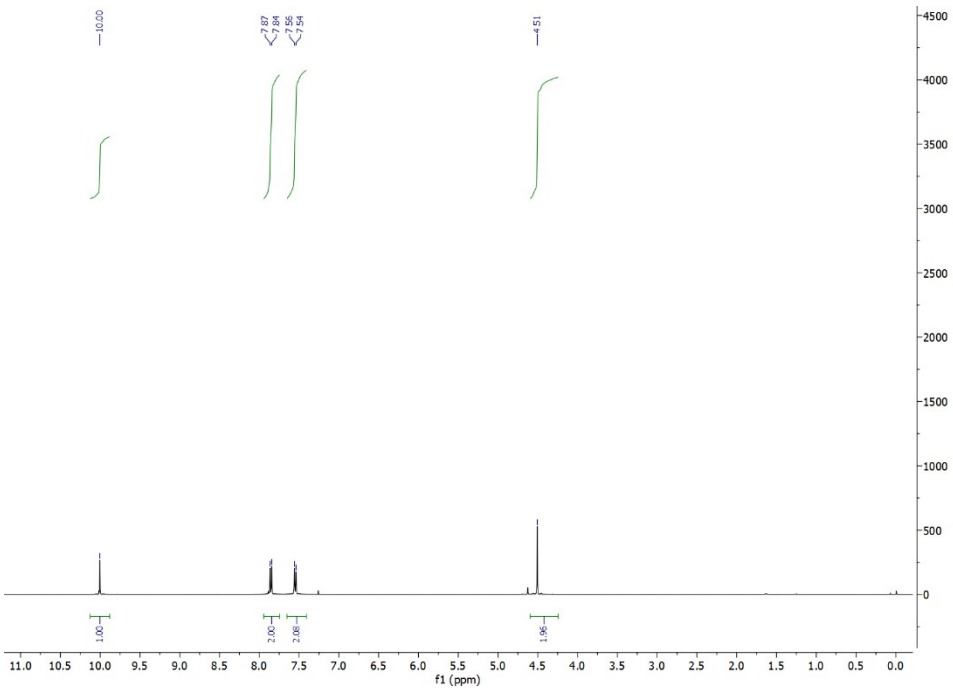


Figure S12. ^{1}H NMR Spectra of compound **2** (400 MHz, $CDCl_3$).

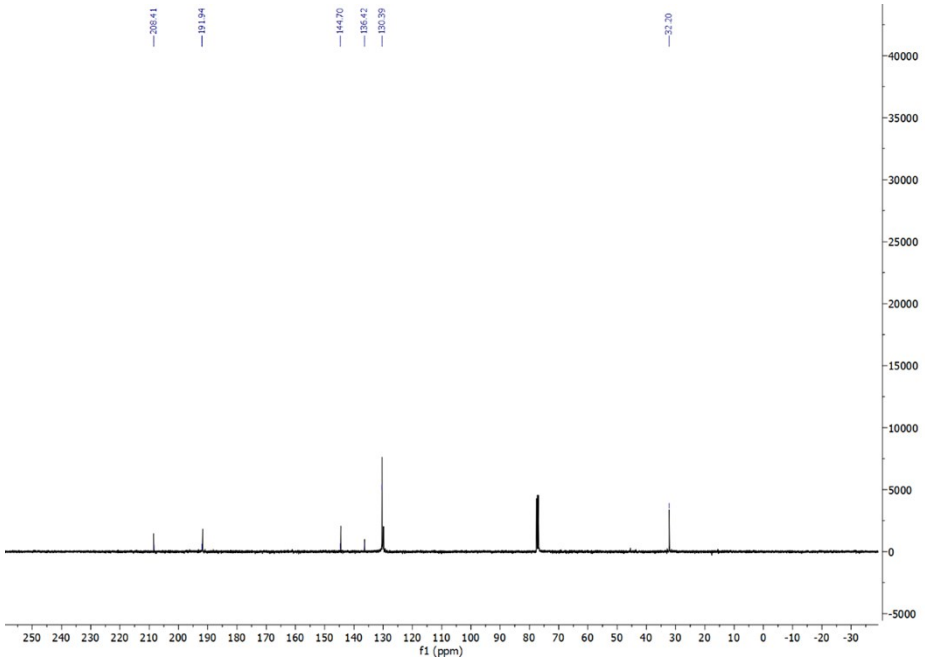


Figure S13. ^{13}C NMR Spectra of compound **2** (400 MHz, $CDCl_3$).

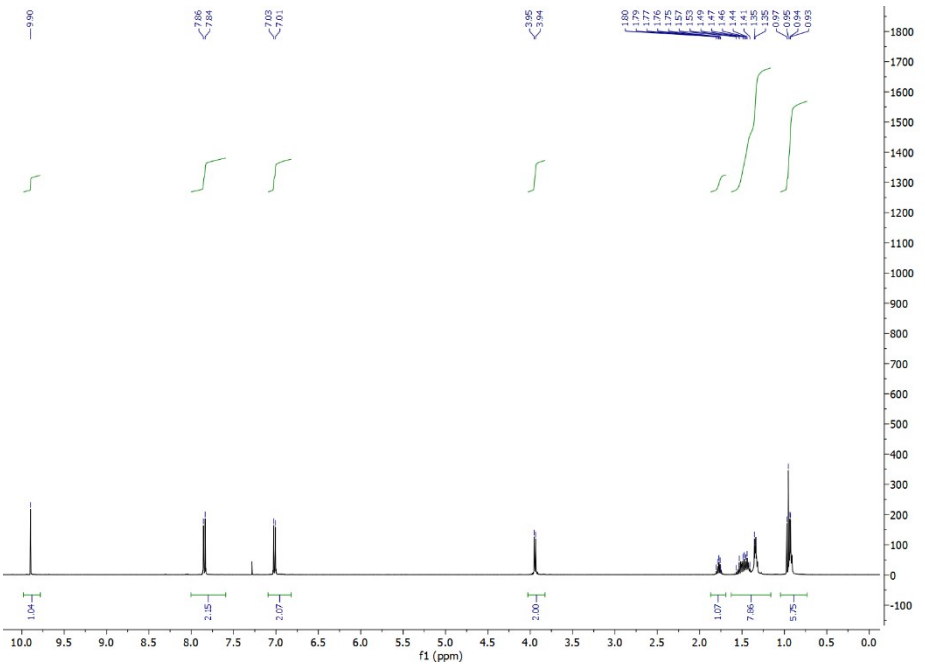


Figure S14. ^{1}H NMR Spectra of compound **3** (400 MHz, $CDCl_3$).

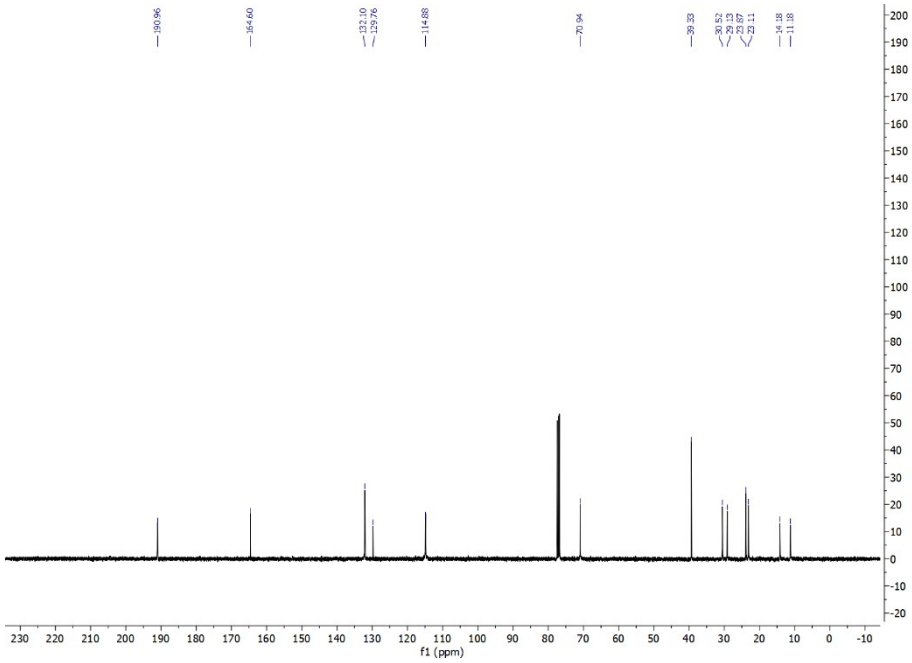


Figure S15. ^{13}C NMR Spectra of compound **3** (400 MHz, $CDCl_3$).

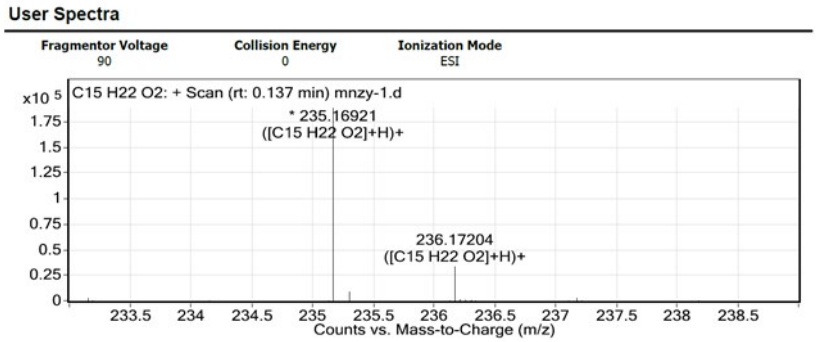


Figure 16. High Resolution ESI-MS of compound **3**.

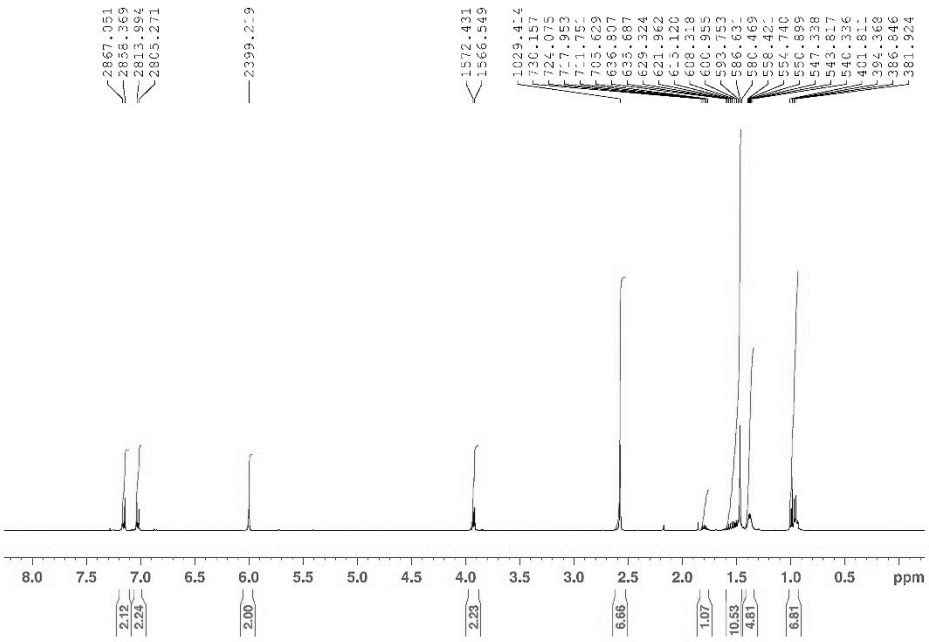


Figure 17. ^{1}H NMR Spectra of compound **4** (400 MHz, $CDCl_3$).

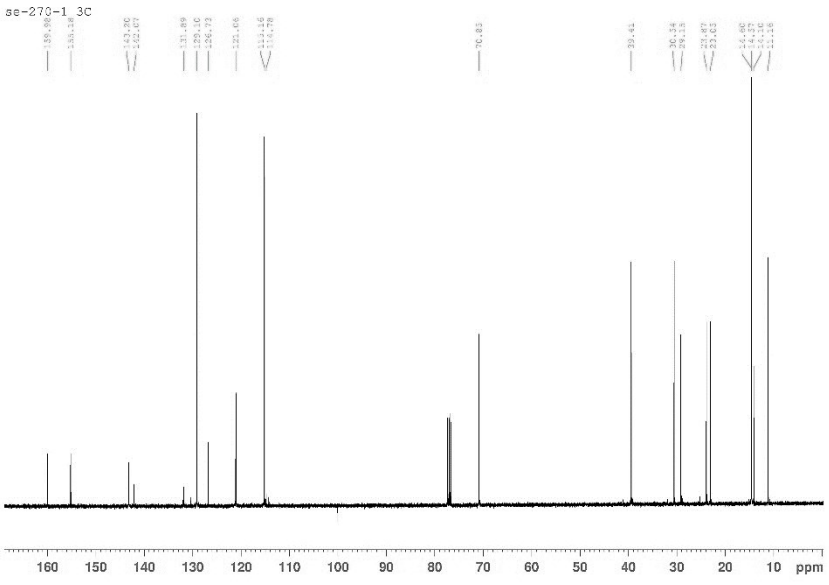


Figure 18. ^{13}C NMR Spectra of compound **4** (400 MHz, $CDCl_3$).

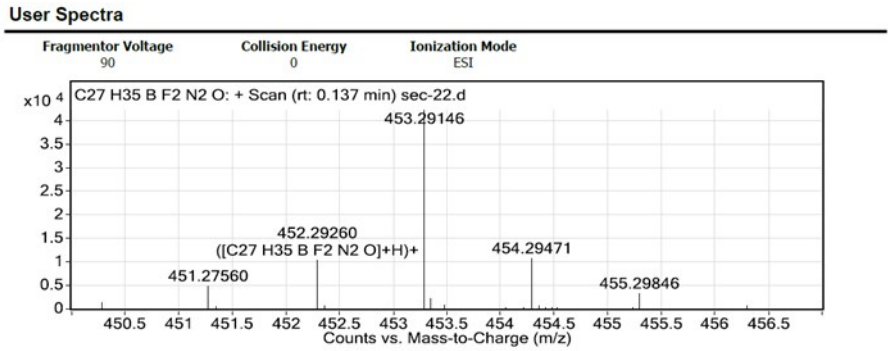


Figure 19. High Resolution ESI-MS of compound **4**.

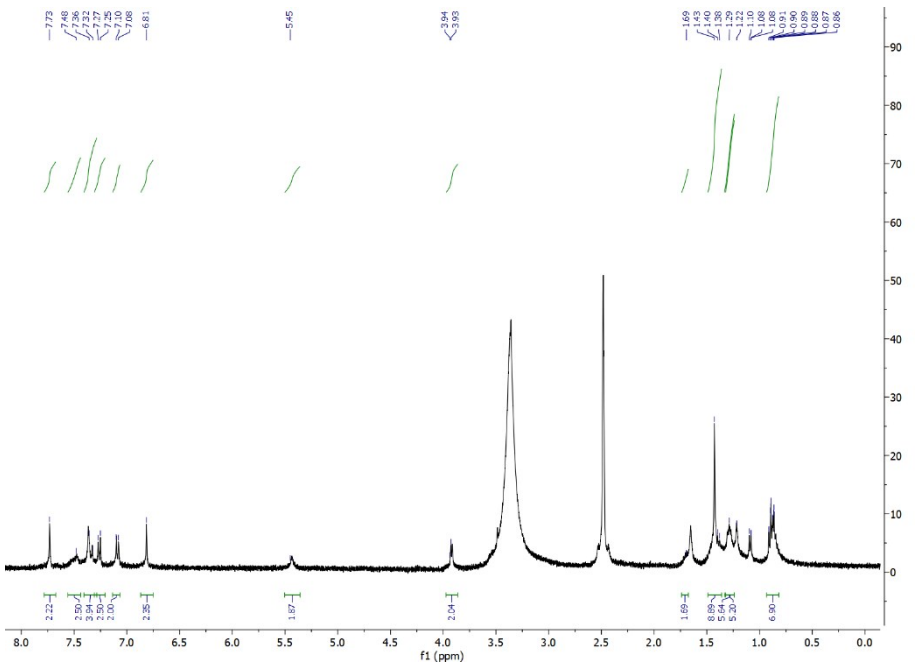


Figure S20. ^{1}H NMR Spectra of **BOD1** (400 MHz, d-DMSO).

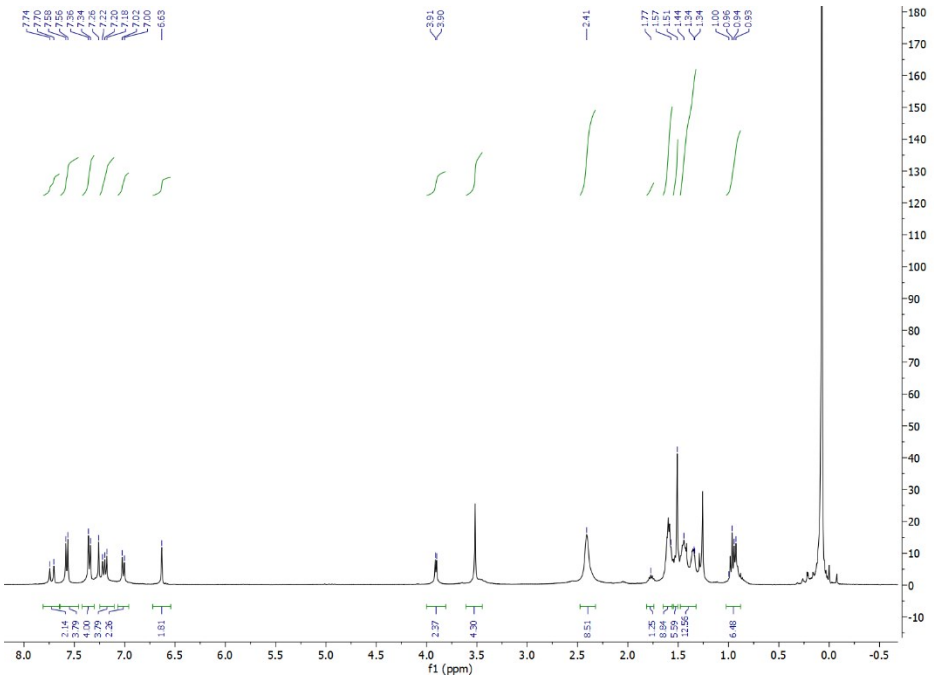


Figure S21. ^{1}H NMR Spectra of **BOD2** (400 MHz, $CDCl_3$).

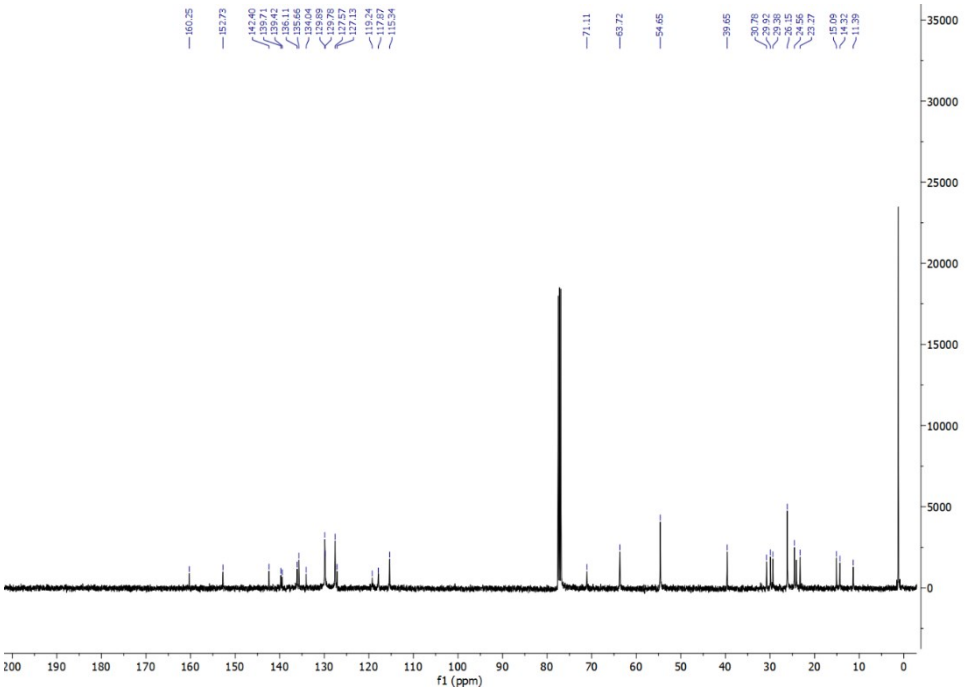


Figure S22. ^{13}C NMR Spectra of **BOD2** (400 MHz, $CDCl_3$).

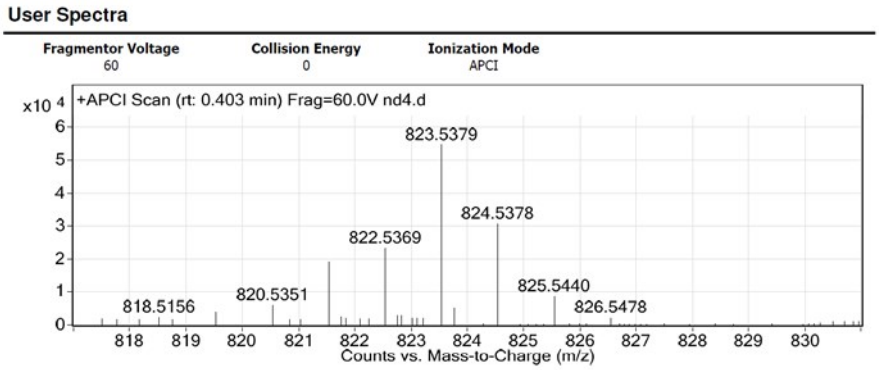


Figure 23. High Resolution ESI-MS of **BOD2**.

5. References

1 H. Rosen, S. J. Klebanoff, *J. Biol. Chem*. 1977, **252**, 4803-4710.