Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

A Fluorescent Probe for Rapid Aqueous Fluoride Detection and Cell Imaging

Bowen Ke,[a] Weixuan Chen,[a] Nanting Ni, Yunfeng Cheng, Chaofeng Dai, Hieu Dinh and Binghe Wang*

Department of Chemistry, Center for Biotechnology and Drug Design, and Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, Georgia 30302-4098,USA.

Tel: 404-413-5545; E-mail: wang@gsu.edu

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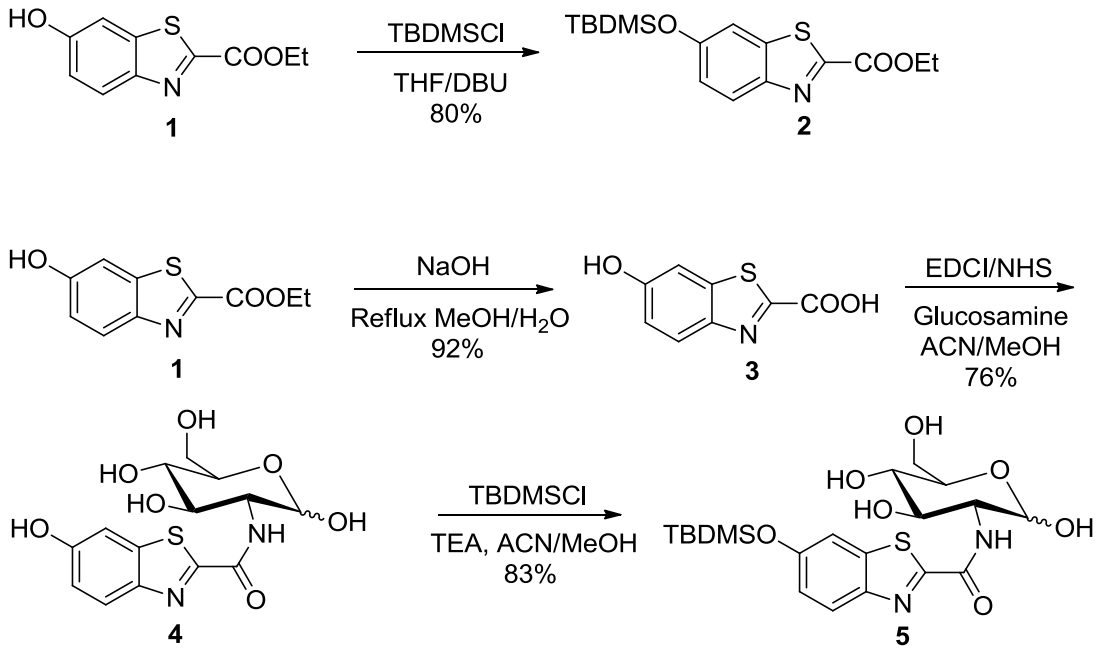
General methods and materials

All chemicals and solvents were reagent grade and anhydrous solvents were obtained from a SG Water solvent purification system. Column chromatography was carried out on flash silica gel (Sorbent 230–400 mesh). TLC analyses were conducted on silica gel plates (Sorbent Silica G UV254). Mass spectral analyses were performed on an ABI API 3200 (ESI-Triple Quadruple). NMR spectra were recorded at ^{1}H (400 MHz) and ^{13}C (100 MHz) on a Bruker instrument. Chemical shifts (δ values) and coupling constants (*J* values) are given in ppm and hertz, respectively, using solvents (^{1}H NMR, ^{13}C NMR) as the internal standard. Fluorescence spectra were recorded on a Shimadzu RF-5301PC fluorometer. Cell imaging was performed on fluorescent microscope (Zeiss Axiovert 200).

Synthesis and Characterization of BBT (2) and BBTGA (5)



Ethyl 6-hydroxybenzothiazole-2-carboxylate (**1**) was synthesized in accordance with a known procedure.[1]

Ethyl 6-((*tert*-butyldimethylsilyl)oxy)benzothiazole-2-carboxylate (2): To a solution of

compound **1** (45 mg, 0.20 mmol) in 25 mL dry THF was added 1.1 equiv. of DBU (34 mg, 0.22 mmol) and TBDMSCl (33 mg, 0.22 mmol). The reaction mixture was stirred at room temperature for 2 h and condensed under reduced pressure. The resulting residue was purified by silica gel flash column chromatography (Hexane/Ethyl Actate = 10:1) to afford **2** as colorless oil (54 mg, 80 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.25 (s, 6H), 1.01 (s, 9H), 1.48 (t, *J* = 7.2 Hz, 3H), 4.54 (q, *J* = 7.2 Hz, 2H), 7.14 (d, *J* = 9.2 Hz, 1H), 7.35 (s, 1H), 8.09 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -4.38, 14.31, 18.22, 25.59, 62.89, 111.39, 121.58, 126.10, 138.38, 148.25, 155.76, 156.18, 160.77.

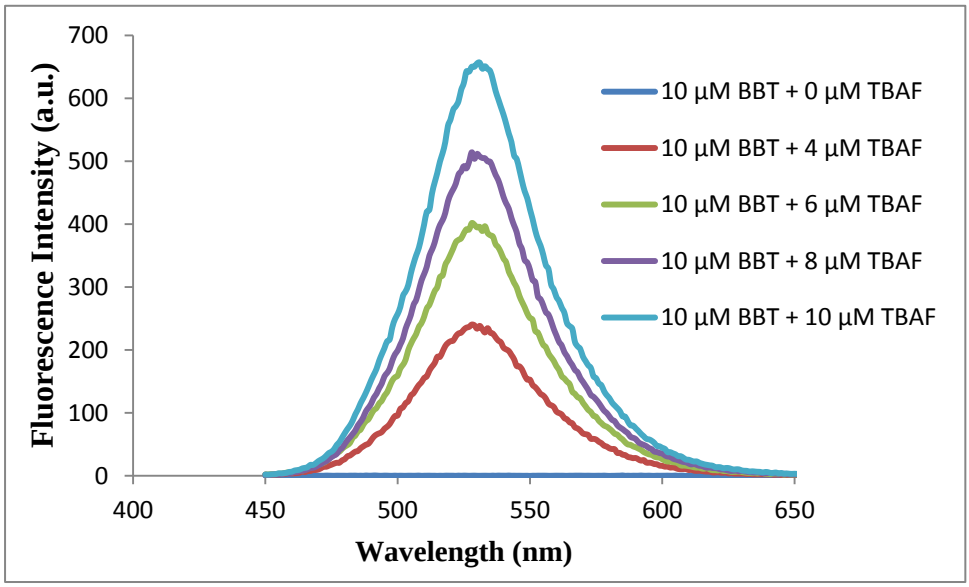
6-Hydroxybenzo[d]thiazole-2-carboxylic acid (3): A stirred mixture of **1** (89 mg, 0.2 mmol) and NaOH (32 mg, 0.2 mmol) in 20 ml ethanol and water (1:1) was refluxed for 5 h. The mixture was concentrated, and the residue was dissolved in water and acidified with 1 N HCl solution. The solution was extracted with EtOAc (20 ml × 3). The combined organic layers were dried in anhydrous Na_2SO_4 to afford acid **3** as yellow powder (72 mg, 92 % yield). mp:150-152°C; ^{1}H NMR (400 MHz, CD_3OD): δ 7.86 (d, *J* = 8.8 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.04 (dd, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 106.16, 115.89, 122.82, 134.95, 152.08, 156.05, 173.84; HRMS (ESI): *m/z* calcd for C_7H_4NOS $[M - COOH]^+$: 150.0014, found: 150.0007.

6-Hydroxybenzo-thiazole-2-carboxylglucosamide (4): Glucosamine hydrochloride (78 mg, 0.36 mmol) was dissolved in MeOH (4 mL) and 5.4 M NaOMe in MeOH (0.067 mL, 0.36 mmol) was added. The reaction mixture was stirred at rt for 1 h. To a solution of compound **3** (70 mg, 0.36 mmol) in CH_3CN (20 mL), 1.2 equiv. of EDCI (82.5 mg, 0.43 mmol), NHS (50 mg, 0.43 mmol) and the glucosamine solution were added. The reaction mixture was stirred at rt overnight, and condensed under reduced pressure. The resulting residue was purified by silica gel flash column chromatography (DCM/MeOH = 6:1) to afford amide **4** (mixture of anomers) as colorless oil (96 mg, 76 % yield). ^{1}H NMR (400 MHz, D_2O): δ 3.44-3.51 (m, 1H), 3.90-3.69 (m, 5H), 4.02 (d, *J* = 3.6 Hz, 1H), 5.31 (d, *J* = 3.2 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 6.99 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, D_2O): δ 54.50, 60.63, 69.97, 70.97, 73.71, 90.89, 113.06, 106.61, 117.09, 124.60, 137.73, 146.03, 155.19, 159.35, 161.57;

HRMS (ESI): *m/z* calcd for $C_{14}H_{15}N_2O_7S$ $[M-H]^+$: 355.0600, found: 355.0615.

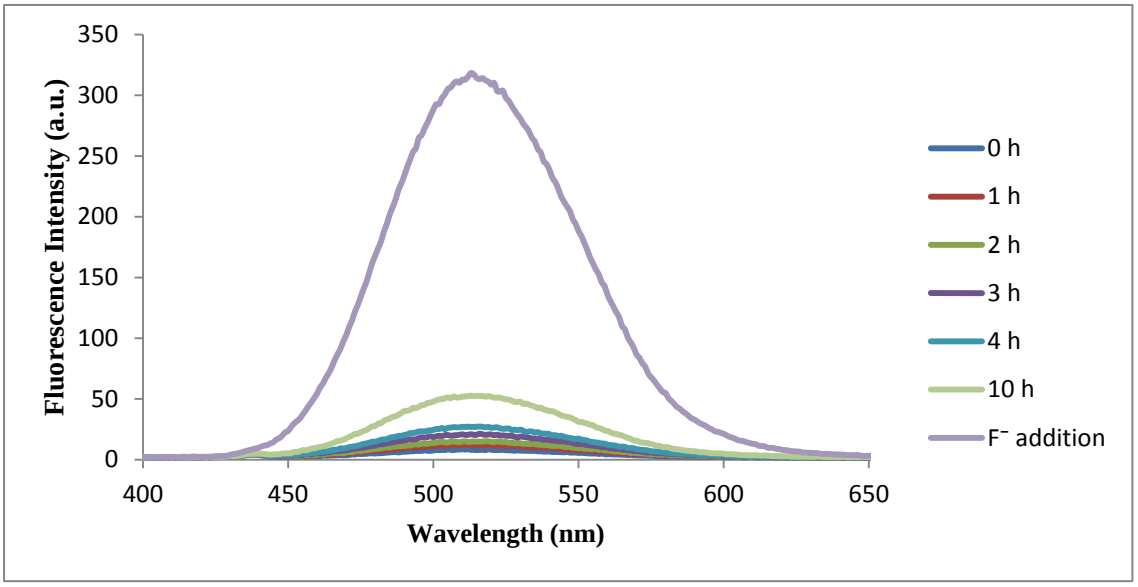
6-((*tert*-Butyldimethylsilyl)oxy)benzo-thiazole-2-carboxylglucosamide (5): To a solution of compound **4** (71 mg, 0.20 mmol) in CH_3CN/MeOH (1:1) were added 1.2 equiv. of TEA (24 mg, 0.24 mmol) and TBDMSCl (36 mg, 0.24 mmol). The reaction mixture was stirred at room temperature overnight, and condensed under reduced pressure. The resulting residue was purified by silica gel flash column chromatography (DCM/MeOH = 10:1) to afford **5** (mixture of anomers) as colorless oil (78 mg, 83 % yield). ^{1}H NMR (400 MHz, CD_3OD): δ 0.28 (s, 6H), 1.04 (s, 9H), 3.40 (d, *J* = 6.0 Hz, 1H), 3.47 (t, *J* = 9.2 Hz, 1H), 3.90-4.06 (m, 4H), 4.08 (dd, *J* = 10.6 Hz, *J* = 3.6 Hz, 1H), 5.26 (d, *J* = 3.2 Hz, 1H), 7.14 (dd, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H), 7.52 (s, 1H), 8.01 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ -5.77, 7.83, 17.70, 54.92, 61.36, 70.82, 71.64, 71.91, 91.19, 111.68, 121.08, 124.73, 138.31, 147.95, 155.17, 160.57, 161.22; HRMS (ESI): *m/z* calcd for $C_{20}H_{31}N_2O_7SSi$ $[M+H]^+$: 471.1621, found: 471.1623.

Fluorescent intensity changes of BBT upon addition of TBAF in THF



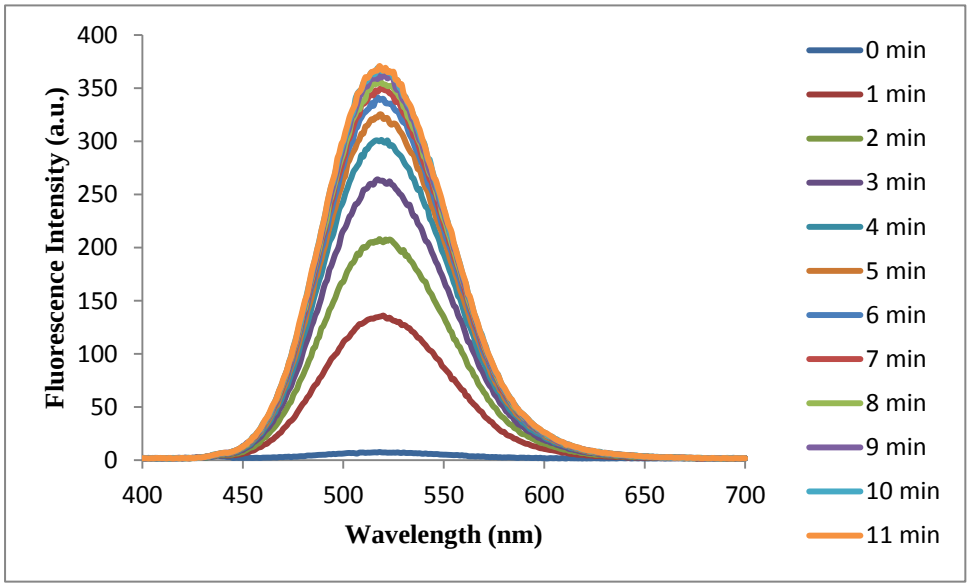
Fluorescence intensity changes (λ_{ex}: 450 nm) of **BBT** (10 μM) in THF at 25 °C. After spiking different concentrations of TBAF in THF, the fluorescent signal increased significantly.

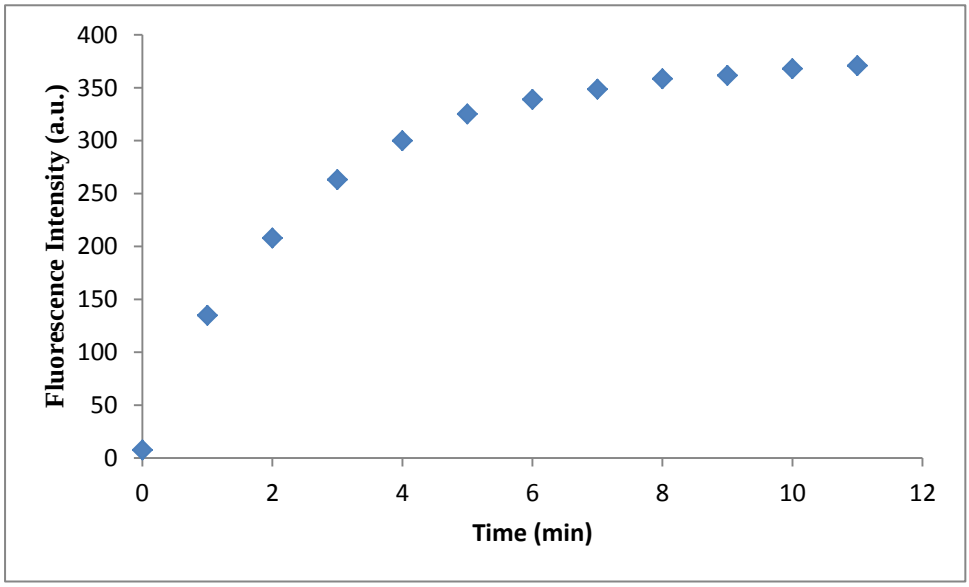
Determination of BBTGA stability in PBS



Fluorescence intensity changes (λ_{ex}: 380 nm) of **BBTGA** (10 μM) during a 10 h period of incubation in PBS (10 mM, pH = 7.4) at 25 °C. After spiking 50 mM NaF, the fluorescent signal increased significantly within 10 min.

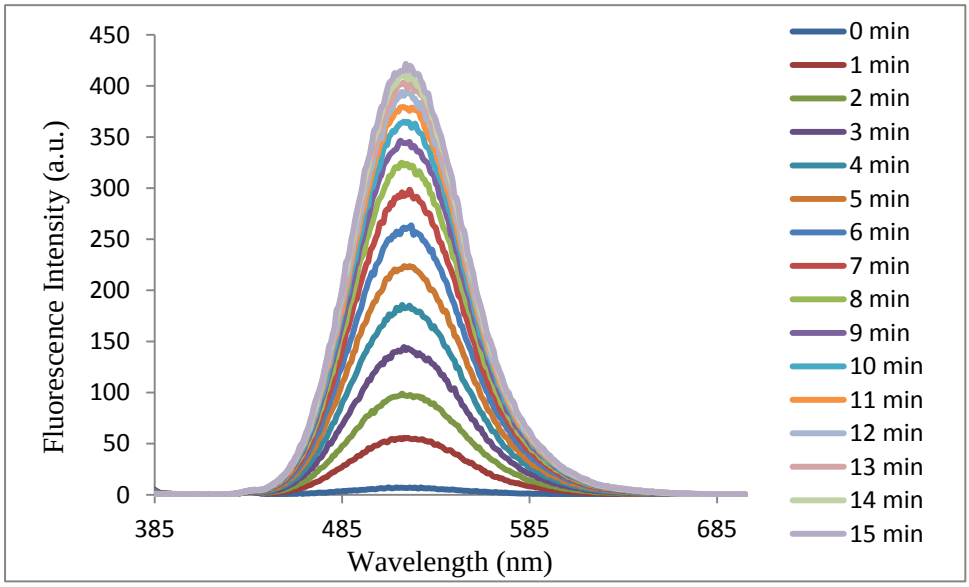
Determination of reaction time





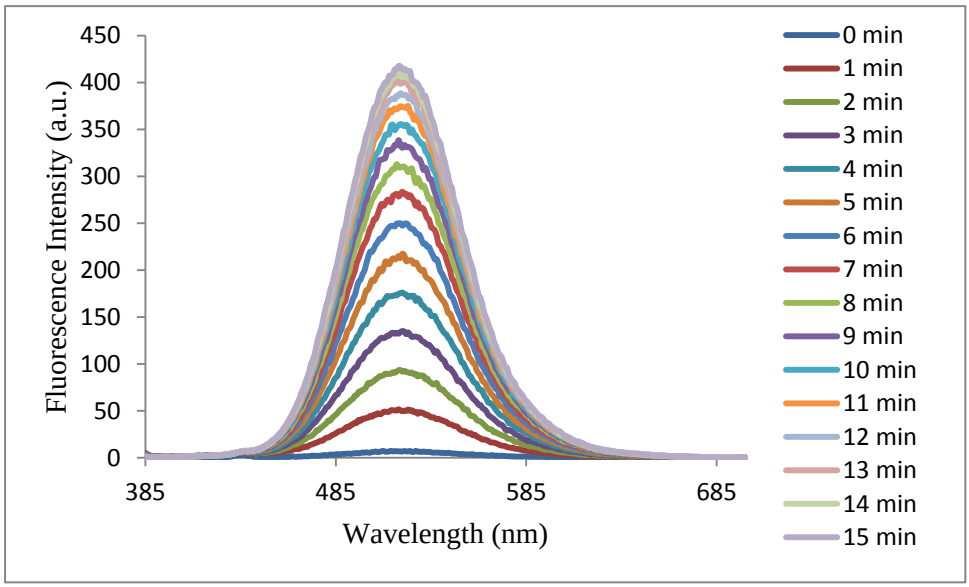
In the presence of 50 mM NaF, the time-dependent fluorescence emission changes (λ_{ex}: 380 nm) of **BBTGA** (10 μM) in 10 mM PBS buffer (pH = 7.4) at 25 °C. The fluorescence emission intensity of **BBTGA** (10 μM) plateaued at 8 min. [Time = 0, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 8 min, 9 min, 10 min, and 11 min.].

Examination of the possible sequestration effect *N*-acetylglucosamine



In the presence of 10 mM NaF, the time-dependent fluorescence emission changes (λ_{ex}: 380 nm) of **BBTGA** (10 μM) in 10 mM PBS buffer (pH = 7.4) at 25 °C. The fluorescence emission intensity of **BBTGA** (10 μM) plateaued at 13 min. [Time = 0, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 8 min, 9 min, 10 min, 11 min, 12 min, 13 min, 14 min and 15

min.].



In the presence of 10 mM NaF and 10 mM N-acetylglycosamine, the time-dependent fluorescence emission changes (λ_{ex}: 380 nm) of **BBTGA** (10 μM) in 10 mM PBS buffer (pH = 7.4) at 25 °C. The fluorescence emission intensity of **BBTGA** (10 μM) plateaued at 13 min. [Time = 0, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 8 min, 9 min, 10 min, 11 min, 12 min, 13 min, 14 min and 15 min.].

Cell culture and MTT assay

Cytotoxicity studies were performed using the MTT assay. KB (human nasopharyngeal epidermal carcinoma) cell lines were cultured in MEM medium (Cellgro) with 10% fetal bovine serum and 1% penicillin/streptomycin. For the cytotoxicity assays, cells were seeded into 96-well plate (3×10^4 in 100 μL per well). Testing compound was dissolved in DMSO to make 10 mM stock solutions. Addition of compounds was performed after adherent cells reached 40-50% confluence. After incubation for 48 h at 37 ^{o}C in humidified atmosphere with 5% CO_2, 10 μL of MTT (5 mg/mL in PBS) was added to each well, which was then incubated for another 4 h. The culture medium was then aspirated and 100 μL of DMSO was added to each well. The 96-well plate was read using Perkin Elmer Victor 3V (multilabel counter model 1420, Perkin Elmer, Boston, MA) for optical density at 490 nm. The test was

performed in triplicates and doxorubicin was used as a positive control. IC_{50} values were estimated based on the concentration that caused 50% of cells death.

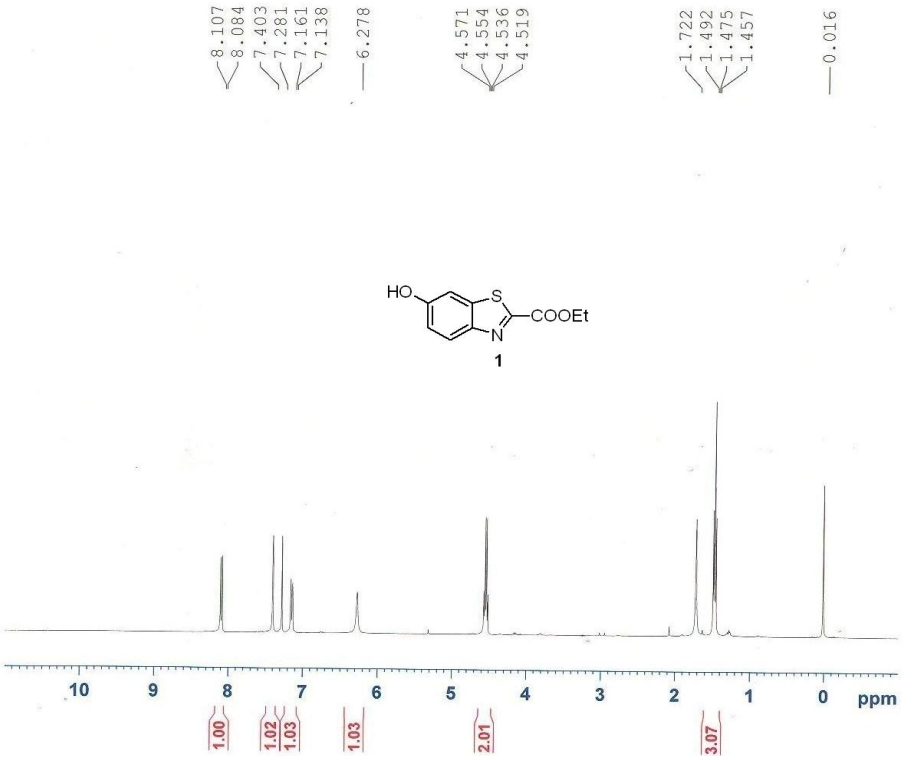
Imaging Studies

KB cells were seeded on a siliconized glass slide in 6-well plate at a density of 3×10^4 cells/well. After 24 h, the cells were treated with the test compound for 24 h at 37 °C in the presence of CO_2 and washed two times with 10 mM PBS (Cellgro). Then, MEM medium (Cellgro, with 10% fetal bovine serum and 1% penicillin/streptomycin) containing 100 uM of NaF was added to the cells. Then the cells were incubated for an additional 2 h. Finally, the glass slide was taken from culture dish and loaded on the fluorescent microscope (Zeiss Axiovert 200). The fluorescent images were taken with a blue filter (excitation: 398 nm, emission: >450nm). Imaging analysis was performed with a Zeiss Axiovert 200.

Reference

1. G. Meroni, M. Rajabi, P. Ciana, A. Maggi and E. Santaniello, *ARKIVOC*, 2010, 53-60.

NMR spectra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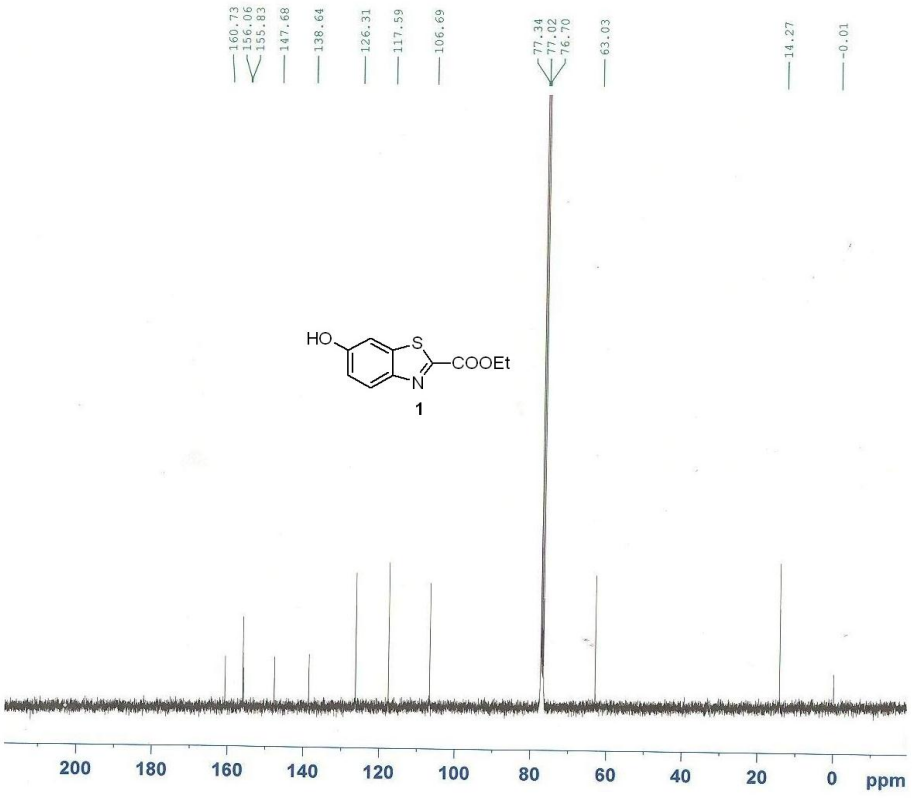
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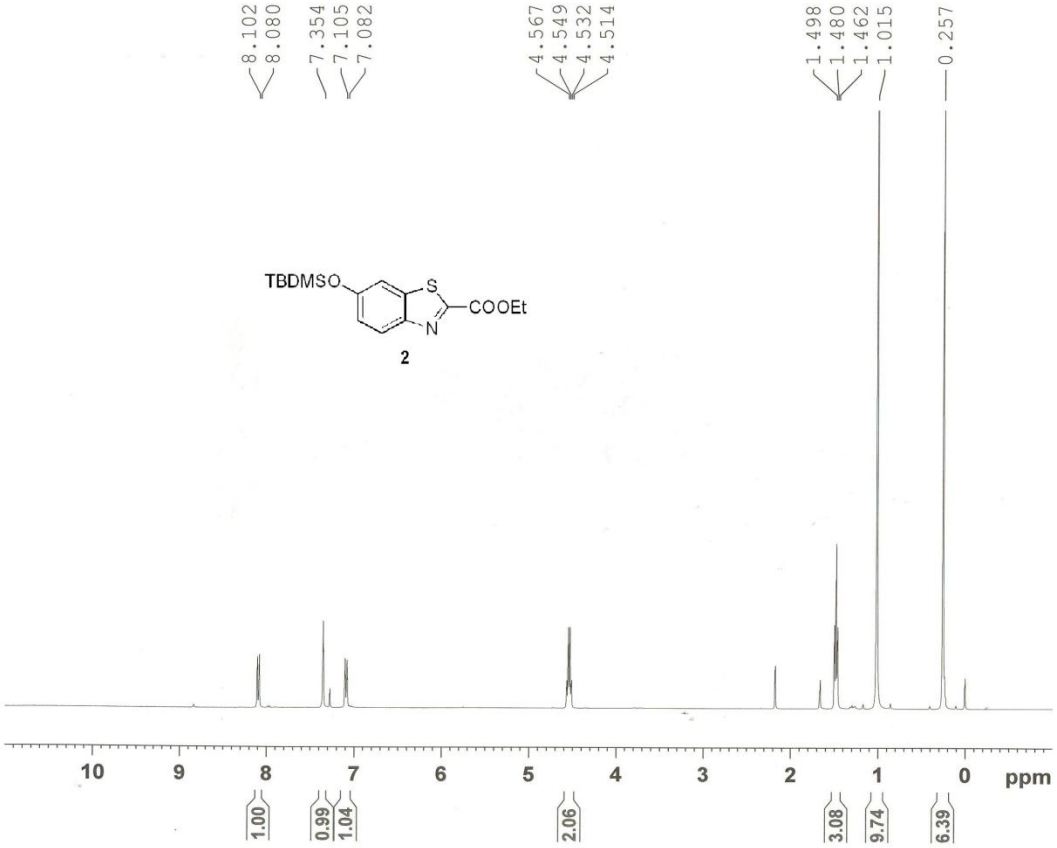
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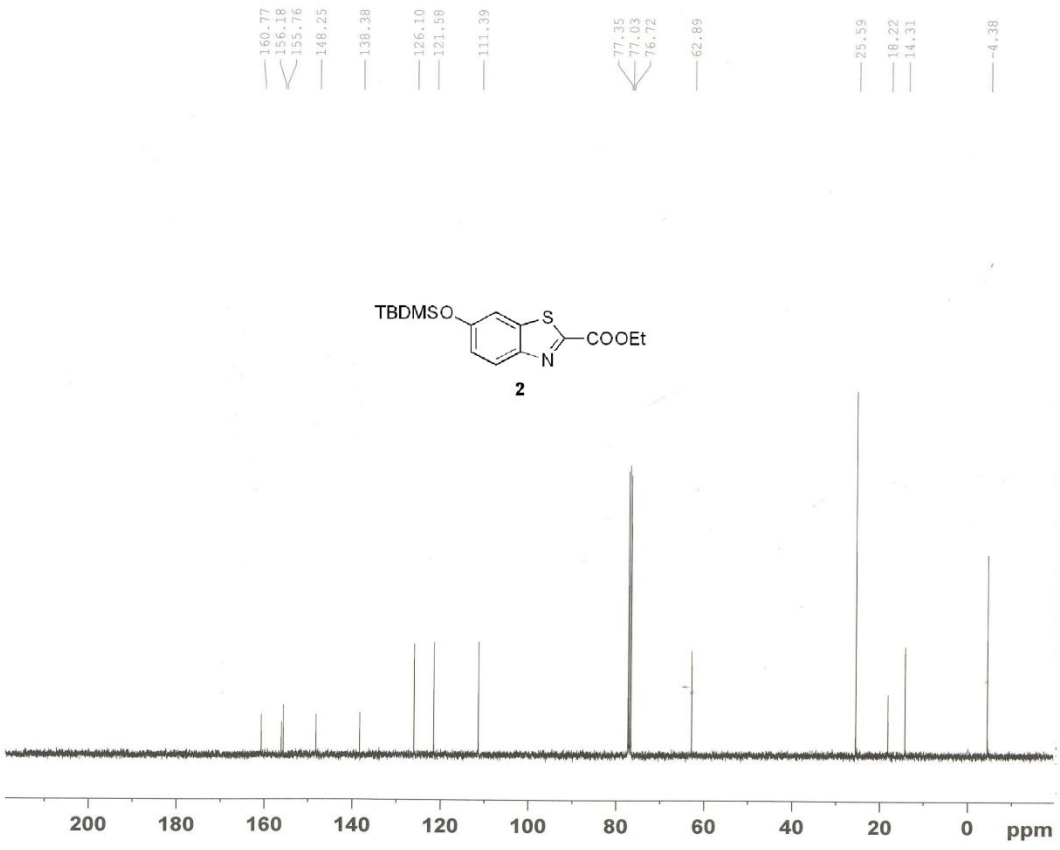
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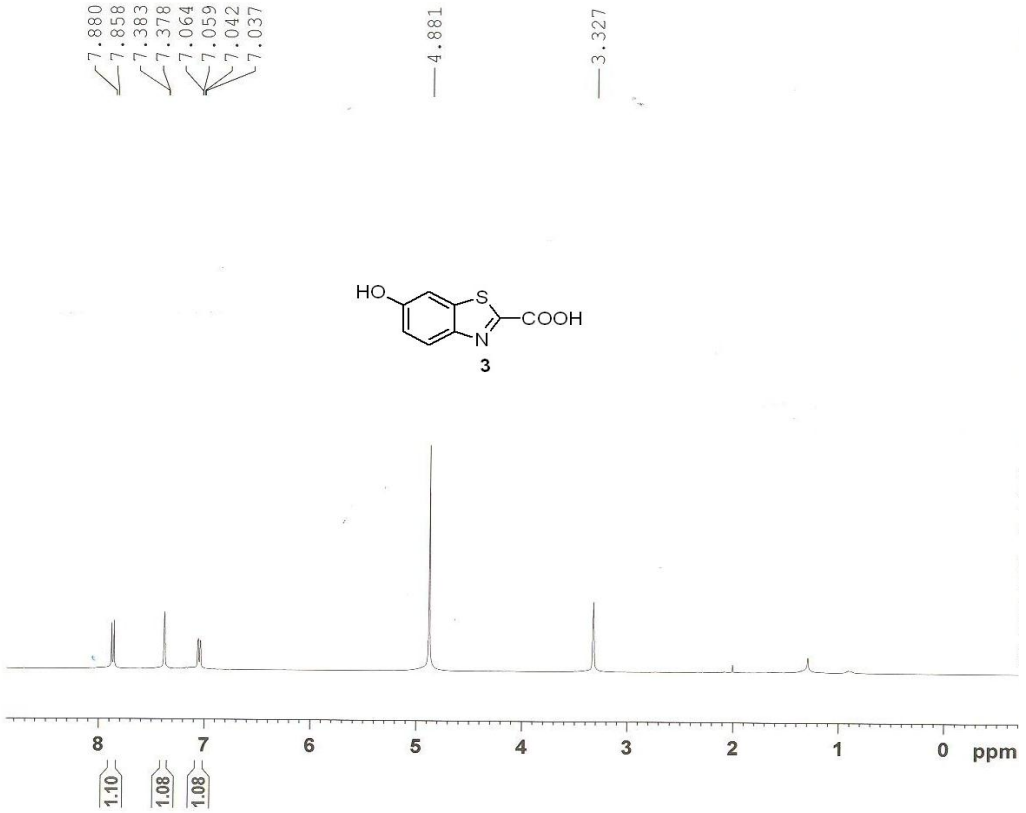
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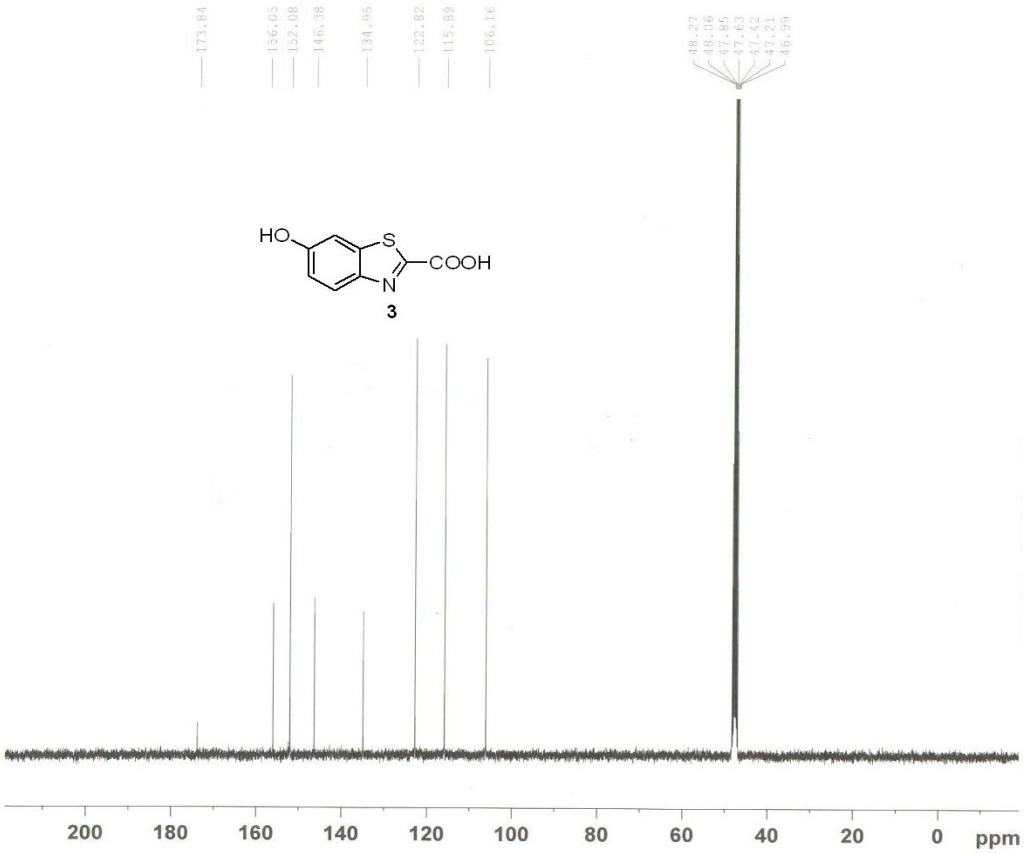
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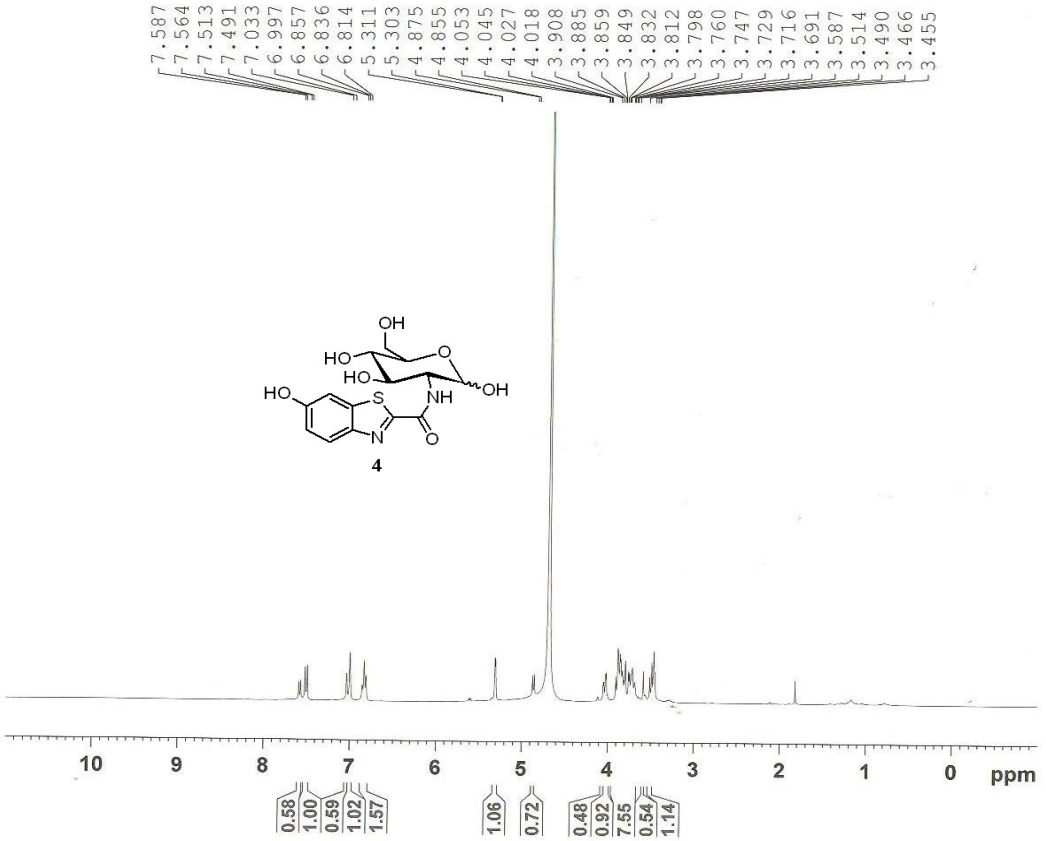
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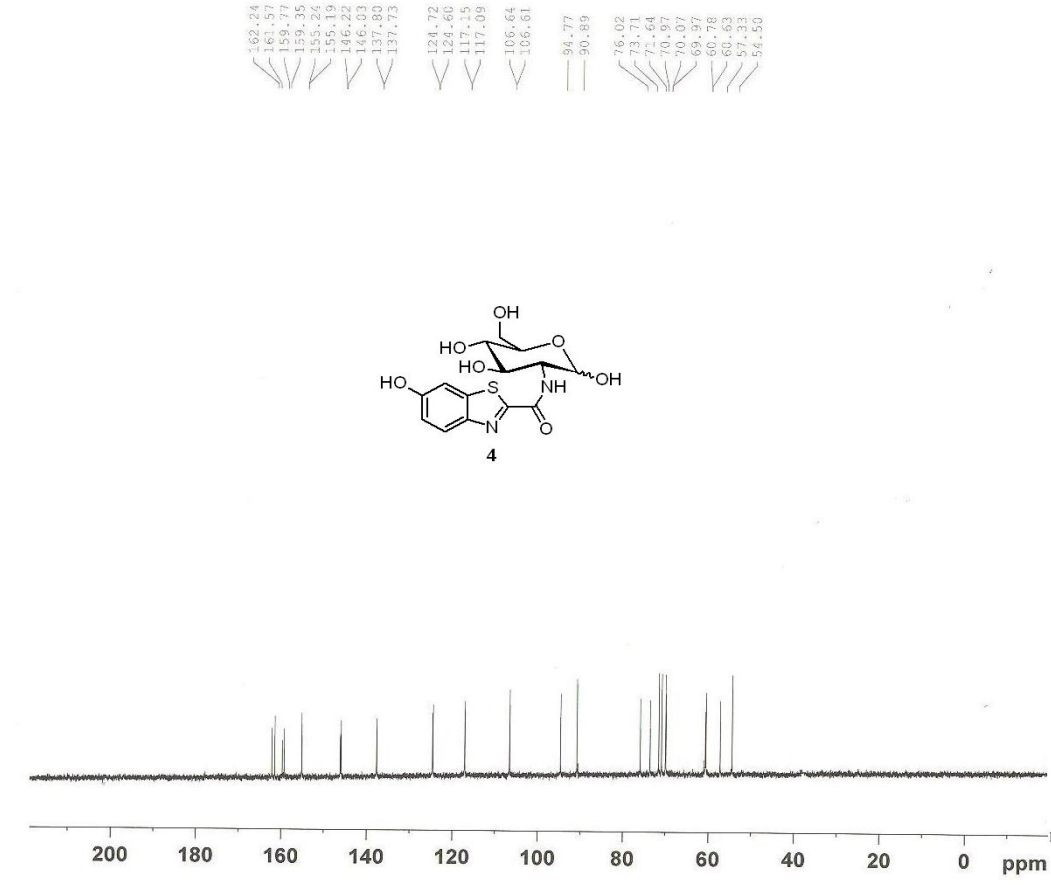
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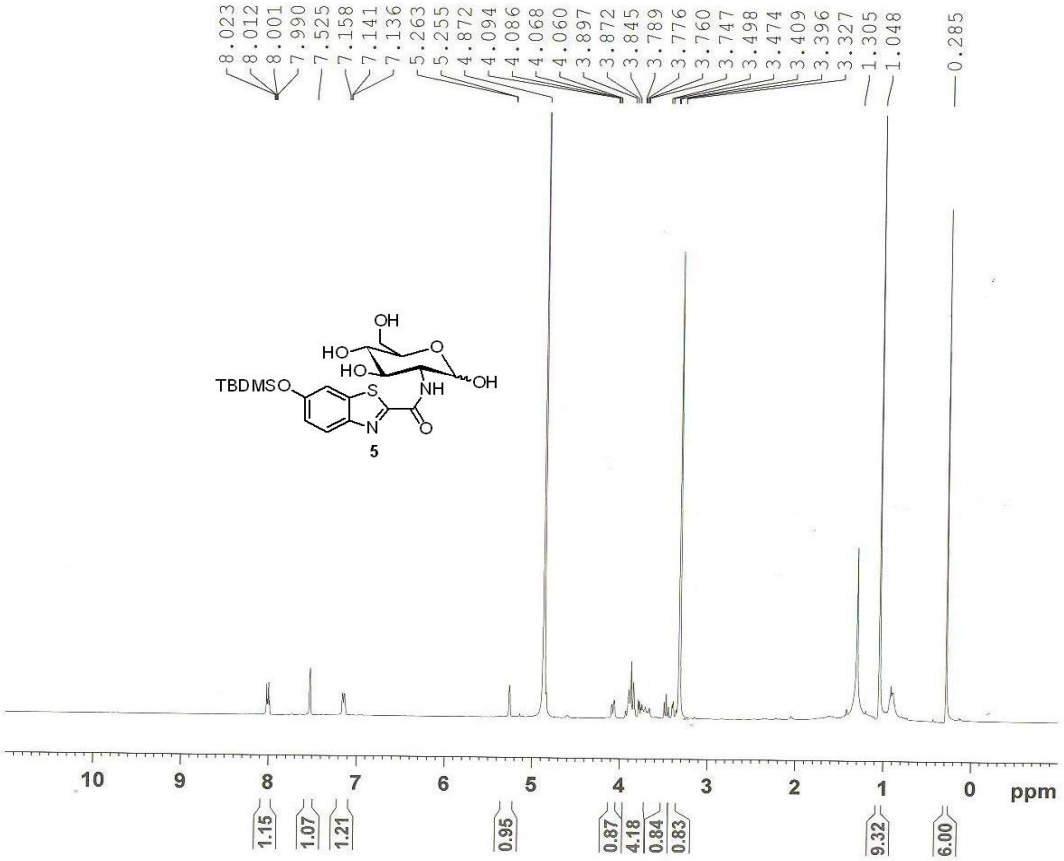
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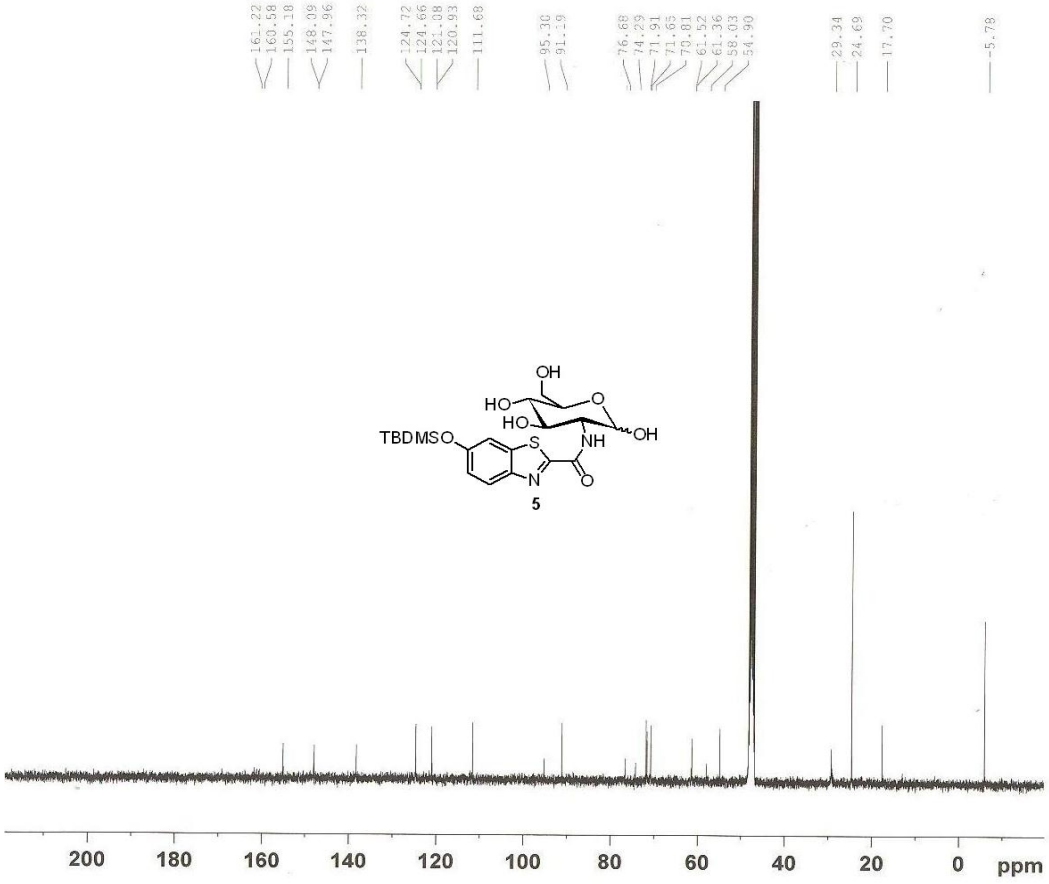
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