Electronic Supplementary Information

Selective photodynamic eradication of senescent cells with a β-galactosidase-activated photosensitiser

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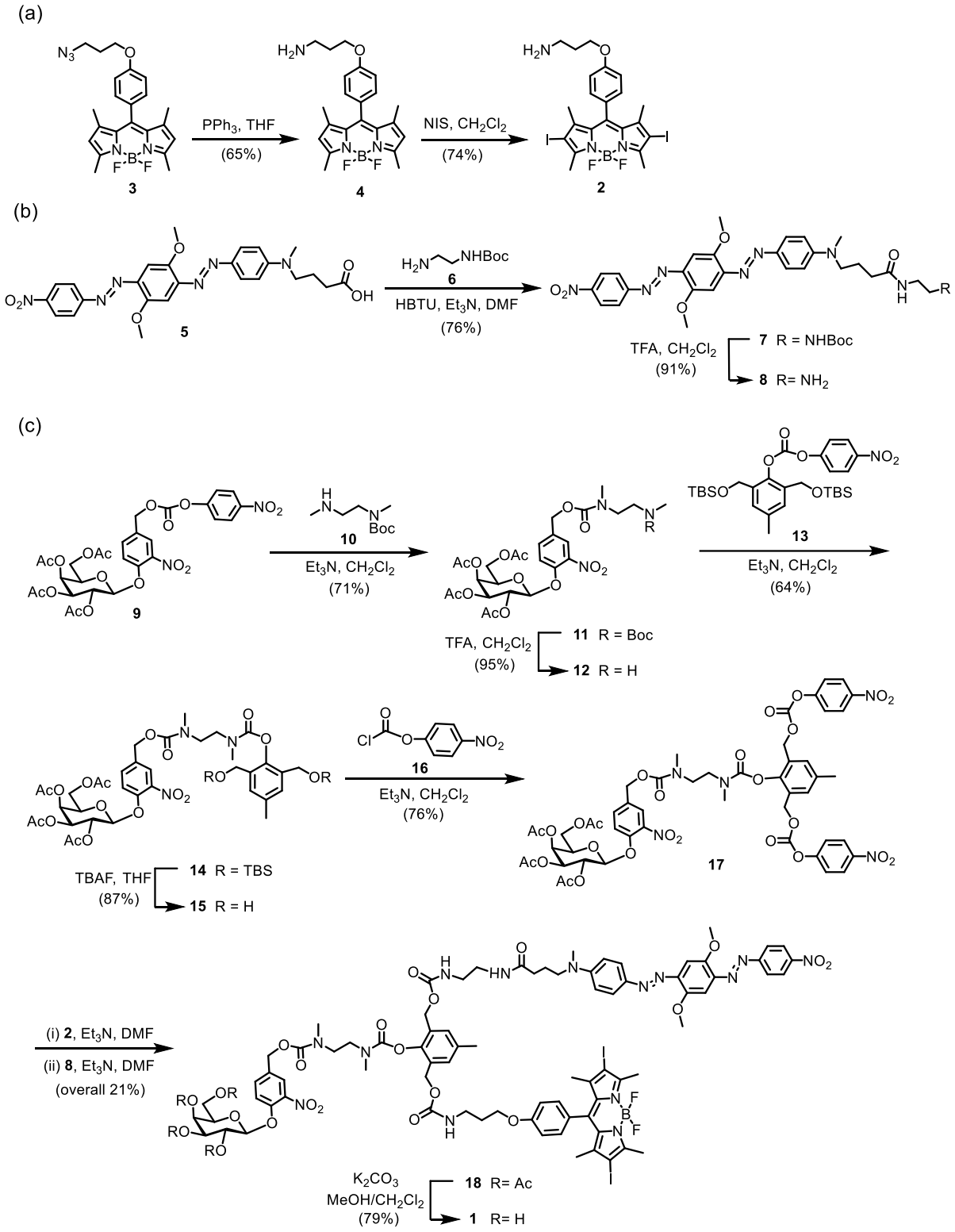
Contents

Synthesis of β-gal-Activated BODIPY-Based Photosensitiser 1

Compound **1** was synthesised through a convergent approach as outlined in Scheme S1. Firstly, reduction of the previously reported azido BODIPY **3**[R1] with triphenylphosphine (PPh_3) in tetrahydrofuran (THF) gave the amino derivative **4**, which was then iodinated with *N*-iodosuccinimide (NIS) in CH_2Cl_2 to afford BODIPY **2** (Scheme S1a). For the BHQ-2 component, compound **5**[R2] was condensed with mono-*tert*-butyloxycarbonyl (Boc)-protected ethylenediamine **6**[R3] in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and Et_3N in *N*,*N*-dimethylformamide (DMF) to afford amide **7**. The Boc protecting group of this compound was then removed upon treatment with trifluoroacetic acid (TFA) to give **8** (Scheme S1b). These two components were then connected to the C1 position of β-D-galactopyranose through an AB_2-type self-immolative linker. As shown in Scheme S1c, treatment of galactose derivative **9**[R4] with mono-Boc-protected *N*,*N*′-dimethyl ethylenediamine **10**[R5] in the presence of Et_3N in CH_2Cl_2 afforded the condensed product **11**. After removing the Boc protecting group, the resulting intermediate **12** underwent condensation with **13**[R6] to give **14**, in which the *tert*-butyldimethylsilyl (TBS) protecting groups were removed upon treatment with tetra-*n*-butylammonium fluoride (TBAF) to give diol **15**. The two hydroxy groups were then activated by the reaction with 4-nitrophenyl chloroformate (**16**) to afford **17**, followed by sequential condensation with **2** and **8** to give **18**. Finally, this compound was hydrolysed under an alkaline condition to remove the acetyl groups to afford the target compound **1**.



Scheme S1. Synthetic routes for the (a) BODIPY-based photosensitiser **2**, (b) BHQ-2-based quencher **8** and (c) conjugate **1**.

Experimental Section

General

DMF, THF and CH_2Cl_2 were dried using an INERT solvent drying system prior to use. All other solvents were of analytical grade and used as received without further purification. All the reactions were performed under an atmosphere of nitrogen and monitored by thin layer chromatography (TLC; Merck pre-coated silica gel 60 F254 plates). Chromatographic purification was performed on silica gel (Macherey-Nagel, 230−400 mesh) with the indicated eluents. Compounds **3**,[R1] **5**,[R2] **6**,[R3] **9**,[R4] **10**[R5] and **13**[R6] were prepared as described.

^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker Avance III 400 spectrometer (^1H, 400 MHz; ^{13}C, 100.6 MHz) or a Bruker Avance III 500 spectrometer (^1H, 500 MHz; ^{13}C, 125.7 MHz) in $CDCl_3$. Spectra were referenced internally using the residual solvent (^1H, $\delta = 7.26$) or solvent (^{13}C, $\delta = 77.2$) resonance(s) relative to $SiMe_4$. Electrospray ionisation (ESI) mass spectra were recorded on a Thermo Finnigan MAT 95 XL mass spectrometer or a Bruker SolariX 9.4 Tesla FTICR mass spectrometer. UV-Vis and steady-state fluorescence spectra were taken on a Shimazu UV-1800 UV-Vis spectrophotometer and a Horiba FluoroMax-4 spectrofluorometer respectively.

Preparation of 4

A mixture of **3** (0.88 g, 2.08 mmol) and PPh_3 (1.64 g, 6.24 mmol) in THF (20 mL) was stirred at room temperature overnight. A small amount of water (5 mL) was then added to the mixture. The resulting mixture was stirred vigorously for further 4 h and then extracted with ethyl acetate (60 mL × 2). The organic layer was dried over anhydrous Na_2SO_4, filtered and

evaporated under reduced pressure. The crude product was purified by column chromatography using CH_2Cl_2/MeOH (5:1, v/v) as the eluent to give **4** (0.54 g, 65%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16 (d, *J* = 8.4 Hz, 2 H, Ar-H), 7.05 (d, *J* = 8.4 Hz, 2 H, Ar-H), 5.95 (s, 2 H, pyrrole-H), 4.17 (t, *J* = 5.6 Hz, 2 H, CH_2), 3.34 (t, *J* = 6.8 Hz, 2 H, CH_2), 2.53 (s, 6 H, CH_3), 2.33-2.39 (m, 2 H, CH_2), 1.39 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 158.9, 155.6, 143.1, 141.5, 131.9, 129.5, 127.9, 121.3, 115.2, 65.5, 38.2, 27.5, 14.8, 14.7. HRMS (ESI): *m/z* calcd for $C_{22}H_{27}BF_2N_3O$ $[M+H]^+$: 398.2214, found 398.2206.

Preparation of 2

A mixture of **4** (0.43 g, 1.08 mmol) and NIS (0.60 g, 2.67 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 6 h. After the complete consumption of **4** indicated by TLC, the mixture was washed with saturated aqueous Na_2SO_3 solution (20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography using CH_2Cl_2/MeOH (4:1, v/v) as the eluent to give **2** (0.52 g, 74%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (d, *J* = 8.8 Hz, 2 H, Ar-H), 7.07 (d, *J* = 8.8 Hz, 2 H, Ar-H), 4.19 (t, *J* = 5.6 Hz, 2 H, CH_2), 3.29 (t, *J* = 6.8 Hz, 2 H, CH_2), 2.63 (s, 6 H, CH_3), 2.28-2.34 (m, 2 H, CH_2), 1.43 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 159.5, 156.9, 145.4, 141.3, 131.8, 129.4, 127.5, 115.5, 85.8, 65.7, 38.4, 28.5, 17.4, 16.2. HRMS (ESI): *m/z* calcd for $C_{22}H_{25}BF_2I_2N_3O$ $[M+H]^+$: 650.0146, found 650.0143.

Preparation of 7

Compound **5** (1.63 g, 3.22 mmol), **6** (1.03 g, 6.43 mmol) and Et_3N (2.2 mL, 15.8 mmol) were mixed in DMF (30 mL) at 0 ℃, and then HBTU (1.22 g, 3.22 mmol) was added. The resulting mixture was allowed to warm to room temperature and then kept stirring overnight. After the complete consumption of **5** indicated by TLC, the mixture was extracted with ethyl acetate (100 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography using hexane/ethyl acetate (6:1, v/v) as the eluent to afford **7** (1.58 g, 76%) as a black solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (d, *J* = 8.8 Hz, 2 H, Ar-H), 8.04 (d, *J* = 8.8 Hz, 2 H, Ar-H), 7.93 (d, *J* = 9.2 Hz, 2 H, Ar-H), 7.50 (s, 1 H, Ar-H), 7.46 (s, 1 H, Ar-H), 6.77 (d, *J* = 9.2 Hz, 2 H, Ar-H), 6.25 (br s, 1 H, NH), 4.90 (br s, 1 H, NH), 4.09 (s, 3 H, OCH_3), 4.05 (s, 3 H, OCH_3), 3.51 (t, *J* = 7.2 Hz, 2 H, CH_2), 3.34-3.38 (m, 2 H, CH_2), 3.20-3.30 (m, 2 H, CH_2), 3.09 (s, 3 H, NCH_3), 2.24 (t, *J* = 7.2 Hz, 2 H, CH_2), 1.96-2.03 (m, 2 H, CH_2), 1.42 (s, 9 H, Boc). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 172.7, 156.6, 153.8, 152.3, 151.1, 148.5, 147.0, 144.6, 142.2, 126.4, 124.9, 123.7, 111.6, 101.1, 100.1, 80.0, 56.9, 51.9, 41.3 40.3, 38.7, 33.4, 28.5, 23.0. HRMS (ESI): *m/z* calcd for $C_{32}H_{41}N_8O_7$ $[M+H]^+$: 649.3092, found 649.3091.

Preparation of 8

TFA (10 mL) was added to a stirred solution of **7** (1.32 g, 2.03 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature for 4 h and then evaporated to dryness under reduced pressure to afford **8** (1.01 g, 91%) as a black powder, which was used directly in the next step without further purification.

Preparation of 11

A mixture of **9** (1.83 g, 2.75 mmol), **10** (0.62 g, 3.29 mmol) and Et_3N (1.2 mL, 8.60 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 4 h. The mixture was washed with saturated brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography using hexane/ethyl acetate (3:1, v/v) as the eluent to afford **11** (1.39 g, 71%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.77 (s, 1 H, Ar-H), 7.50 (d, *J* = 8.5 Hz, 1 H, Ar-H), 7.32 (d, *J* = 8.5 Hz, 1 H, Ar-H), 5.52 (dd, *J* = 8.0, 10.5 Hz, 1 H, galactose-H), 5.44-5.45 (m, 1 H, galactose-H), 5.05-5.10 (m, 4 H, galactose-H and $ArCH_2$), 4.21-4.24 (m, 1 H, galactose-H), 4.12-4.16 (m, 1 H, galactose-H), 4.05-4.10 (m, 1 H, galactose-H), 3.32-3.37 (m, 4 H, NCH_2), 2.93 (s, 3 H, $BocNCH_3$), 2.85 and 2.79 (s, 3 H total, NCH_3 of the rotamers), 2.17 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 1.41 (s, 9 H, Boc). $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 169.4, 155.9, 155.6, 155.5, 148.9, 141.2, 133.5, 133.2, 131.7, 125.1, 124.8, 124.6, 119.7, 100.7, 79.8, 79.6, 79.4, 71.4, 70.5, 67.8, 66.7, 65.4, 65.2, 61.3, 60.4, 47.3, 47.0, 46.8, 46.5, 45.9, 35.3, 35.1, 34.8, 34.5, 28.4, 21.0, 20.7, 20.6, 20.5, 14.2 (the occurrence of extra signals are due to the presence of rotamers). HRMS (ESI): *m/z* calcd for $C_{31}H_{43}N_3NaO_{16}$ $[M+Na]^+$: 736.2535, found 736.2526.

Preparation of 12

TFA (10 mL) was added to a stirred solution of **11** (1.25 g, 1.75 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature for 2 h, and then evaporated to dryness under reduced pressure to afford **12** (1.02 g, 95%) as a colourless oil, which was used directly in the next step

without further purification.

Preparation of 14

A mixture of **12** (0.93 g, 1.52 mmol), **13** (1.01 g, 1.80 mmol) and Et_3N (0.65 mL, 4.66 mmol) in CH_2Cl_2 (40 mL) was stirred at room temperature overnight. It was then washed with saturated brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtrated and evaporated *in vacuo*. The residue was purified by column chromatography using hexane/ethyl acetate (4:1, v/v) as the eluent to afford **14** (1.01 g, 64%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79-7.81 (m, 1 H, Ar-H), 7.51-7.53 (m, 1 H, Ar-H), 7.31-7.34 (m, 1 H, Ar-H), 7.18 (s, 2 H, Ar-H), 5.52-5.56 (m, 1 H, galactose-H), 5.45-5.46 (m, 1 H, galactose-H), 5.01-5.15 (m, 4 H, galactose-H and $ArCH_2$), 4.60 (br. s, 4 H, $ArCH_2$), 4.20-4.26 (m, 1 H, galactose-H), 4.14-4.18 (m, 1 H, galactose-H), 4.03-4.05 (m, 1 H, galactose-H), 3.47-3.61 (m, 4 H, NCH_2), 2.96-3.14 (m, 6 H, NCH_3 of the rotamers), 2.34 (s, 3 H, $ArCH_3$), 2.19 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 0.91 (s, 18 H, Si^{t}Bu), 0.06 (s, 12 H, $Si(CH_3)_2$). ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.5, 156.1, 155.7, 154.3, 153.9, 149.1, 143.2, 143.1, 142.9, 141.4, 135.4, 133.5, 133.4, 133.3, 133.2, 127.4, 127.3, 127.2, 126.2, 124.7, 119.9, 115.7, 100.9, 71.5, 70.7, 67.9, 66.8, 65.7, 65.5, 61.4, 60.6, 60.4, 60.3, 48.1, 47.9, 47.6, 47.4, 47.2, 46.9, 46.5, 36.0, 35.9, 35.8, 35.5, 35.4, 34.9, 26.1, 21.4, 20.8, 20.7, 18.5, -5.1 (the occurrence of extra signals are due to the presence of rotamers). HRMS (ESI): *m/z* calcd for $C_{48}H_{73}N_3NaO_{18}Si_2$ $[M+Na]^+$: 1058.4319, found 1058.4314.

Preparation of 15

A mixture of **14** (0.83 g, 0.80 mmol) and TBAF (1 M, 5 mL) in THF (15 mL) was stirred at room temperature for 6 h. The mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography using CH_2Cl_2/MeOH (6:1, v/v) as the eluent to afford **15** (0.56 g, 87%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74-7.79 (m, 1 H, Ar-H), 7.47-7.52 (m, 1 H, Ar-H), 7.30 (d, *J* = 8.5 Hz, 1 H, Ar-H), 7.17-7.19 (m, 2 H, Ar-H of the rotamers), 5.49-5.51 (m, 1 H, galactose-H), 5.42-5.43 (m, 1 H, galactose-H), 4.99-5.11 (m, 4 H, galactose-H and $ArCH_2$), 4.47 and 4.40 (s, 4 H total, $ArCH_2$ of the rotamers), 4.20-4.22 (m, 1 H, galactose-H), 4.11-4.15 (m, 1 H, galactose-H), 4.00-4.03 (m, 1 H, galactose-H), 3.47-3.64 (m, 4 H, NCH_2), 2.94, 3.00, 3.08 and 3.15 (s, 6 H total, NCH_3 of the rotamers), 2.49 (br s, 2 H, OH), 2.31 (s, 3 H, $ArCH_3$), 2.16 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 1.99 (s, 3 H, OAc). ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 170.2, 169.5, 156.5, 156.2, 156.1, 156.0, 155.9, 155.7, 155.6, 149.2, 149.1, 149.0, 145.2, 145.0, 144.9, 141.2, 141.1, 141.0, 136.4, 136.3, 134.0, 133.7, 133.6, 133.4, 133.3, 133.0, 132.8, 132.7, 132.5, 130.6, 130.3, 130.2, 130.1, 125.1, 124.9, 124.8, 124.7, 119.8, 119.7, 100.8, 100.7, 100.6, 71.4, 70.6, 67.9, 66.8, 65.9, 65.8, 65.7, 65.6, 61.4, 60.7, 60.5, 47.7, 47.4, 47.3, 47.1, 46.9, 46.7, 46.0, 36.0, 35.7, 35.5, 35.2, 35.1, 35.0, 34.3, 20.9, 20.7 (the occurrence of extra signals are due to the presence of rotamers). HRMS (ESI): *m/z* calcd for $C_{36}H_{45}N_3NaO_{18}$ $[M+Na]^+$: 830.2590, found 830.2588.

Preparation of 17

To a solution of **15** (0.41 g, 0.51 mmol) in CH_2Cl_2 (20 mL), 4-nitrophenyl chloroformate (**16**) (0.24 g, 1.19 mmol) and Et_3N (0.43 mL, 3.08 mmol) were added. The mixture was stirred at room temperature overnight, and then it was washed with an aqueous brine (10 mL). The

organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography using hexane/ethyl acetate (3:1, v/v) as the eluent to afford **17** (0.44 g, 76%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, *J* = 8.4 Hz, 4 H, Ar-H), 7.79-7.84 (m, 1 H, Ar-H), 7.50-7.54 (m, 1 H, Ar-H), 7.29-7.40 (m, 7 H, Ar-H), 5.54-5.58 (m, 1 H, galactose-H), 5.48-5.49 (m, 1 H, galactose-H), 5.27 and 5.25 (s, 2 H total, $ArCH_2$ of the rotamers), 5.10-5.17 (m, 4 H, $ArCH_2$ of the rotamers), 5.06 (d, *J* = 8.0 Hz, 1 H, galactose-H), 4.49-4.51 (m, 1 H, galactose-H), 4.25-4.29 (m, 1 H, galactose-H), 4.16-4.19 (m, 1 H, galactose-H), 4.08-4.10 (m, 1 H, galactose-H), 3.52-3.67 (m, 4 H, NCH_2), 2.96-3.24 (m, 6 H total, NCH_3 of the rotamers), 2.41 and 2.39 (s, 3 H total, $ArCH_3$ of the rotamers), 2.21 (s, 3 H, OAc), 2.14 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.04 (s, 3 H, OAc). ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 170.4, 170.3, 169.5, 155.6, 154.3, 152.5, 149.1, 146.1, 145.8, 145.6, 141.4, 136.6, 133.1, 132.3, 132.0, 131.5, 130.8, 128.0, 125.4, 125.1, 124.7, 122.0, 119.9, 100.9, 71.5, 70.7, 67.9, 66.8, 66.2, 65.5, 61.4, 47.6, 47.1, 46.6, 46.2, 41.4, 36.0, 35.7, 35.4, 35.1, 34.7, 34.5, 21.0, 20.8, 20.7 (the occurrence of extra signals are due to the presence of rotamers). HRMS (ESI): *m/z* calcd for $C_{50}H_{51}N_5NaO_{26}$ $[M+Na]^+$: 1160.2714, found 1160.2706.

Preparation of 18

To a mixture of **17** (149 mg, 0.13 mmol) and Et_3N (55 μL, 0.39 mmol) in DMF (5 mL), a solution of **2** (85 mg, 0.13 mmol) in DMF (10 mL) was added in dropwise over a period of 30 min. The mixture was stirred at room temperature for 20 h, and then a solution of **8** (144 mg, 0.26 mmol) in DMF (2 mL) was added. Stirring was continued at room temperature overnight.

The resulting mixture was extracted with ethyl acetate (40 mL × 2), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography using hexane/ethyl acetate (3:1, v/v) as the eluent to afford **18** (56 mg, 21%) as a violet solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, *J* = 9.0 Hz, 2 H, Ar-H), 8.04 (d, *J* = 9.0 Hz, 2 H, Ar-H), 7.92 (d, *J* = 9.0 Hz, 2 H, Ar-H), 7.75-7.84 (m, 1 H, Ar-H), 7.50 (s, 2 H, Ar-H), 7.46 (d, *J* = 2.0 Hz, 1 H, Ar-H), 7.32 (d, *J* = 9.0 Hz, 1 H, Ar-H), 7.17 (br s, 2 H, Ar-H), 7.11 (d, *J* = 8.5 Hz, 2 H, Ar-H), 6.96 (br s, 2 H, Ar-H), 6.74 (d, *J* = 8.5 Hz, 2 H, Ar-H), 6.44 (br s, 1 H, NH), 6.28- 6.33 (m, 1 H, NH), 6.02 (s, 2 H, $ArCH_2$), 5.63 (br s, 1 H, NH), 5.51-5.53 (m, 1 H, galactose-H), 5.46 (s, 2 H, $ArCH_2$), 5.21-5.30 (m, 2 H, galactose-H), 4.97-5.10 (m, 10 H, $ArCH_2$ and CH_2), 4.11-4.24 (m, 3 H, galactose-H), 4.09 (s, 3 H, OCH_3), 4.03 (s, 3 H, OCH_3), 3.73-3.74 (m, 1 H, galactose-H), 3.28-3.50 (m, 10 H, CH_2), 2.94-3.07 (m, 9 H total, NCH_3 of the rotamers), 2.62 (s, 3 H, CH_3), 2.55 (s, 3 H, CH_3), 2.34 and 2.30 (s, 3 H total, $ArCH_3$ of the rotamers), 2.18 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.85-1.91 (m, 2 H, CH_2), 1.40 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ 170.5, 170.3, 170.2, 169.5, 156.6, 153.8, 152.3, 151.0, 148.5, 146.9, 144.5, 142.2, 129.3, 126.4, 124.9, 123.6, 122.4, 115.3, 111.5, 101.0, 100.9, 100.1, 84.3, 71.5, 70.6, 67.9, 66.8, 65.9, 61.3, 56.9, 51.8, 47.0, 38.7, 35.6, 29.6, 22.8, 21.0, 20.8, 20.7, 17.3, 17.0, 16.2, 15.9, 15.0, 14.9 (the occurrence of extra signals are due to the presence of rotamers). HRMS (ESI): *m/z* calcd for $C_{87}H_{98}BF_2I_2N_{14}O_{26}$ $[M+H]^+$: 2057.4935, found 2057.4941.

Preparation of 1

A mixture of **18** (29 mg, 14 μmol) and K_2CO_3 (10 mg, 0.07 mmol) in CH_2Cl_2/MeOH (4:1, v/v,

10 mL) was stirred at room temperature for 8 h. The solvent was then evaporated under reduced pressure and the residue was purified by column chromatography using CH_2Cl_2/ MeOH (6:1, v/v) as the eluent to afford **1** (21 mg, 79%) as a violet solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, *J* = 8.5 Hz, 2 H, Ar-H), 8.04 (d, *J* = 8.5 Hz, 2 H, Ar-H), 7.92 (d, *J* = 8.5 Hz, 2 H, Ar-H), 7.76-7.88 (m, 1 H, Ar-H), 7.46-7.54 (m, 3 H, Ar-H), 7.33 (s, 1 H, Ar-H), 7.11-7.20 (m, 4 H, Ar-H), 6.97 (br s, 2 H, Ar-H), 6.75 (br s, 2 H, Ar-H), 6.38 (br s, 2 H, NH), 6.02 (s, 2 H, ArCH_2), 5.30 (s, 4 H, ArCH_2), 5.07-5.10 (m, 3 H), 4.81-4.97 (m, 4 H), 4.09 (s, 3 H, OCH_3), 4.03 (s, 3 H, OCH_3), 3.95-3.99 (m, 3 H), 3.84 (br s, 2 H), 3.16-3.65 (m, 12 H), 2.91-3.04 (m, 9 H total, NCH_3 of the rotamers), 2.62 (s, 3 H, CH_3), 2.55 (s, 3 H, CH_3), 2.29 (s, 3 H, ArCH_3), 2.17-2.01 (m, 5 H), 1.90-1.86 (m, 3 H), 1.40 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ 159.7, 159.5, 157.8, 157.3, 156.7, 156.5, 154.5, 153.7, 152.3, 151.0, 149.1, 148.5, 146.8, 145.4, 145.3, 144.5, 143.3, 142.2, 141.6, 141.5, 141.3, 133.6, 133.5, 133.3, 132.9, 132.3, 131.8, 131.4, 130.1, 129.3, 127.4, 127.2, 127.0, 126.3, 124.9, 124.7, 123.6, 122.4, 119.8, 115.4, 115.3, 111.5, 101.1, 100.8, 100.1, 85.7, 84.3, 71.5, 70.6, 68.1, 68.0, 66.8, 66.0, 65.4, 63.6, 63.4, 61.4, 56.9, 56.8, 51.7, 41.3, 40.0, 38.7, 38.6, 34.4, 33.1, 31.1, 30.4, 29.8, 29.5, 25.7, 22.9, 17.3, 17.0, 16.1, 15.9, 15.0, 14.9 (the occurrence of extra signals are due to the presence of rotamers). HRMS (ESI): *m/z* calcd for $C_{79}H_{90}BF_2I_2N_{14}O_{22}$ $[M+H]^+$: 1889.4511, found 1889.4508.

Determination of fluorescence quantum yields

The fluorescence quantum yields (Φ_F) were determined by the equation:[R7]

$$\Phi_{F(sample)} = \left(\frac{F_{sample}}{F_{ref}}\right)\left(\frac{A_{ref}}{A_{sample}}\right)\left(\frac{n^2_{sample}}{n^2_{ref}}\right)\Phi_{F(ref)}$$

where *F*, *A* and *n* are the measured fluorescence (area under the emission peak), the absorbance

at the excitation wavelength (490 nm) and the refractive index of the solvent respectively. Fluorescein in NaOH solution (0.1 M, pH = 13, Φ_F = 0.925) was used as the reference.[R8] To minimise reabsorption of radiation by the ground-state species, the emission spectra were obtained in very dilute solutions of which the absorbance at 490 nm was about 0.03.

Measurement of singlet oxygen generation

1,3-Diphenylisobenzofuran (DPBF) was used as the singlet oxygen scavenger.[R9] A solution of DPBF (80 μM) and BODIPY **1** or **2** (2 μM) in PBS (pH = 7.4) with 0.1% Tween 80 (v/v) with or without β-galactosidase (10 unit mL^{-1}) was irradiated with red light from a 300 W halogen lamp after passing through a water tank for cooling and a colour glass filter with a cut-on wavelength at 515 nm (Newport). The absorption maximum of DPBF at 417 nm was monitored along with the irradiation time.

Cell lines and culture conditions

HeLa human cervical cancer cells (ATCC, no. CCL-2) and HT29 human colorectal adenocarcinoma cells (ATCC, no. HTB38) were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, cat. no. 23400-021) supplemented with fetal bovine serum (10%) and penicillin-streptomycin (100 unit mL^{-1} and 100 μg mL^{-1} respectively). The cells were cultured in a humidified incubator under a 5% CO_2 atmosphere at 37 °C.

Intracellular fluorescence imaging

Approximately 2 × 10^5 native and senescent HeLa and HT29 cells in the medium (2 mL) were

seeded on a confocal dish respectively and incubated at 37 °C overnight in a humidified 5% CO_2 atmosphere. After being rinsed with phosphate-buffered saline (PBS) (1 mL) twice, the cells were incubated with C_{12}FDG (25 μM) in the medium for 35 min or **1** (4 μM) in the medium with 0.05% Tween 80 (v/v) for 2 h. After removal of the medium, the cells were rinsed with PBS (1 mL) twice, and then replenished with 1 mL of Hank's balanced salt solution (HBSS). The cells were then examined using a confocal microscope (Leica TCS SP8 high-speed imaging system) with a helium-neon 488 nm laser to monitor the fluorescence at 500-650 nm. The images were digitised and analysed using a Leica Application Suite X software.

Flow cytometric analysis

After the treatments mentioned above, the cells were harvested by 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) (Invitrogen, 0.2 mL). The activity of trypsin was quenched with a serum-containing medium (0.8 mL), and the mixture was centrifuged at 1500 rpm for 3 min at room temperature. The pellet was then rinsed with PBS (1 mL) and subject to centrifugation. The cells were then suspended in PBS (1 mL) and the fluorescence intensities of cells were measured using a BD FACSVerse flow cytometer (Becton Dickinson) with 10^4 cells counted in each sample. The compounds were excited by an argon laser at 488 nm and the emitted fluorescence was monitored at 500-560 nm. The data collected were analysed using the BD FAC-Suite. All experiments were performed in triplicate.

Annexin V - red fluorescent protein (RFP) staining for intracellular fluorescence imaging

A stock solution of **1** (40 μM) was prepared by dissolving the compound (40 nmol) in

dimethylsulfoxide (DMSO) (20 μL) in the presence of Tween 80 (5 μL), followed by dilution with RPMI 1640 medium (975 μL). Photodynamic treatment was performed on the cells seeded on confocal dishes 24 h prior to the assay. The cells were incubated with **1** (10 μM) for 2 h. After being rinsed with PBS (1mL) twice, the cells were illuminated with a halogen lamp (300 W) with a yellow glass filter (Newport, cut-on at 515 nm) for 20 min at room temperature. The fluence rate used was 25.5 mW cm^{-2}, giving a total fluence of 30.6 J cm^{-2}. After illumination, the cells were incubated in dark at 37 ℃ under 5% CO_2 for 20 h. The cells were then rinsed with PBS (1 mL) twice and suspended in 1 mL of binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH = 7.4) containing annexin V-RFP (10 μL) for 15 min. After removal of the binding buffer, the cells were rinsed with PBS (1 mL) twice and replenished with 1 mL of HBSS. The cells were then examined using a confocal microscope (Leica TCS SP8 high-speed imaging system) with a 552 nm laser to monitor the fluorescence of RFP at 640-680 nm. This region can exclude the fluorescence emitted by **1**.

Annexin V-RFP staining for flow cytometric analysis

A stock solution of **1** (40 μM) was prepared by dissolving the compound (40 nmol) in DMSO (20 μL) in the presence of Tween 80 (5 μL), followed by dilution with RPMI 1640 medium (975 μL). Photodynamic treatment was performed on the cells seeded on 96-well plates 24 h prior to the assay. The cells were incubated with **1** at various concentrations (0, 2.5, 5, and 10 μM) for 2 h. After being rinsed with PBS (1 mL) twice, the cells were illuminated with a halogen lamp (300 W) with a yellow glass filter (Newport, cut-on at 515 nm) for 20 min at room temperature. The fluence rate used was 25.5 mW cm^{-2}, giving a total fluence of 30.6 J

cm^{-2}. After illumination, the cells were incubated in dark at 37 ℃ under 5% CO_2 for 20 h. The cells were then rinsed with PBS (1 mL) twice and suspended in 1 mL of binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH = 7.4) containing annexin V-RFP (10 μL) for 15 min. After removal of the binding buffer, the cells were rinsed with PBS (1 mL) twice and harvested by 0.25% trypsin-EDTA (Invitrogen, 0.2 mL). The activity of trypsin was quenched with a serum-containing medium (0.8 mL), and the mixture was centrifuged at 1500 rpm for 3 min at room temperature. The pellet was then washed with PBS (1 mL) twice and subject to centrifugation. The cells were then suspended in PBS (1 mL) and the fluorescence intensities of the cells were measured using a BD FACSVerse flow cytometer (Becton Dickinson) with 10^4 cells counted in each sample. RFP was excited by an argon laser at 488 nm and its fluorescence was monitored at 640-680 nm, in which the fluorescence emitted by **1** can be excluded. The data collected were analysed using the BD FAC-Suite. All experiments were performed in triplicate.

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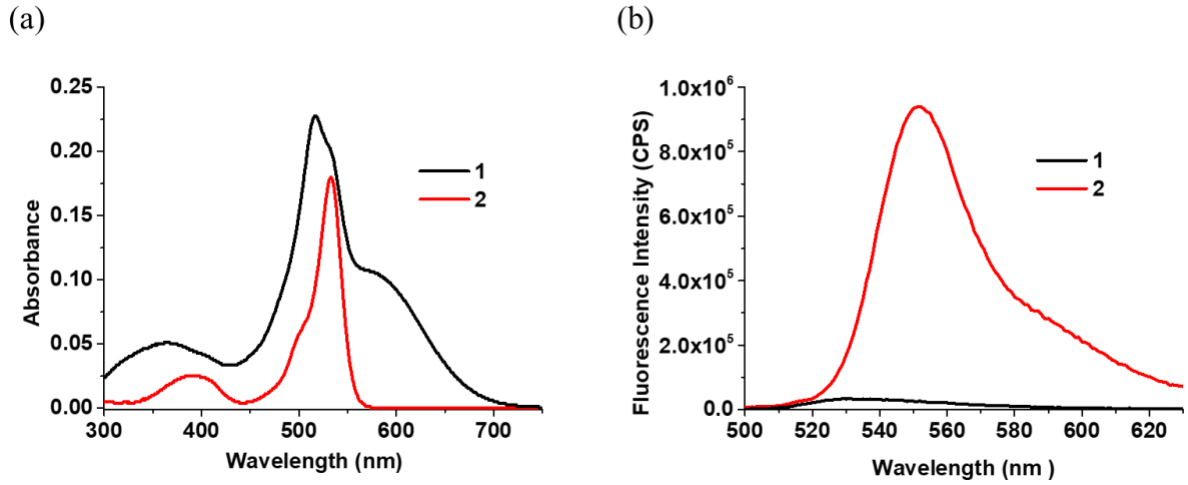


Fig. S1 (a) Electronic absorption and (b) fluorescence (λ_{ex} = 490 nm) spectra of **1** and **2** (2 μM) in PBS (pH = 7.4) with 0.1% Tween 80 (v/v).

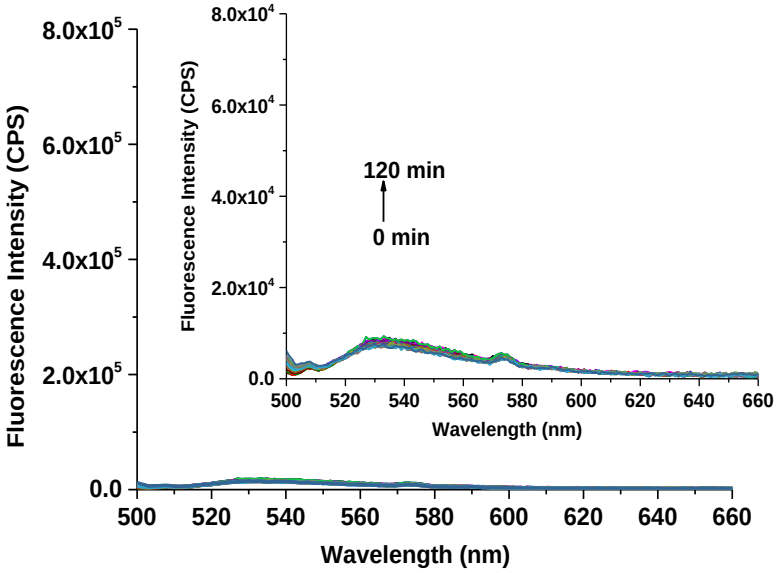


Fig. S2 Change in fluorescence spectrum (λ_{ex} = 490 nm) of **1** (2 μM) in the absence of β-gal in PBS (pH = 7.4) with 0.1% Tween 80 (v/v) over a period of 2 h. The inset shows the enlarged spectra.

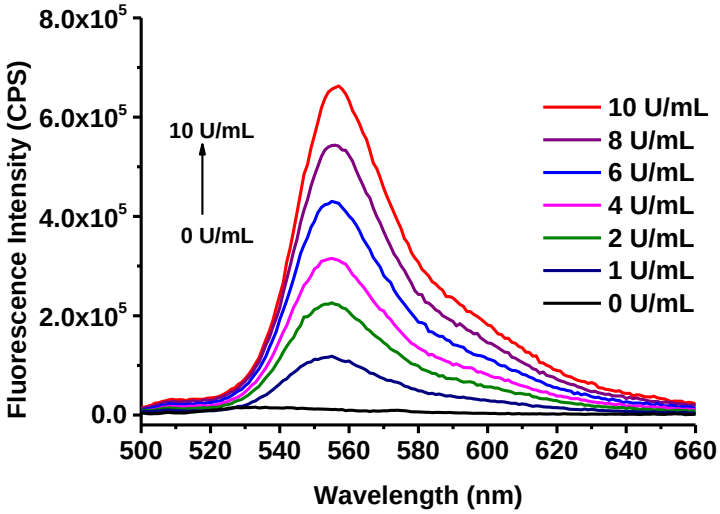


Fig. S3 Fluorescence spectra (λ_{ex} = 490 nm) of **1** (2 μM) after the treatment with various concentrations of β-gal (0-10 unit mL^{-1}) in PBS (pH = 7.4) with 0.1% Tween 80 (v/v) for 2 h.

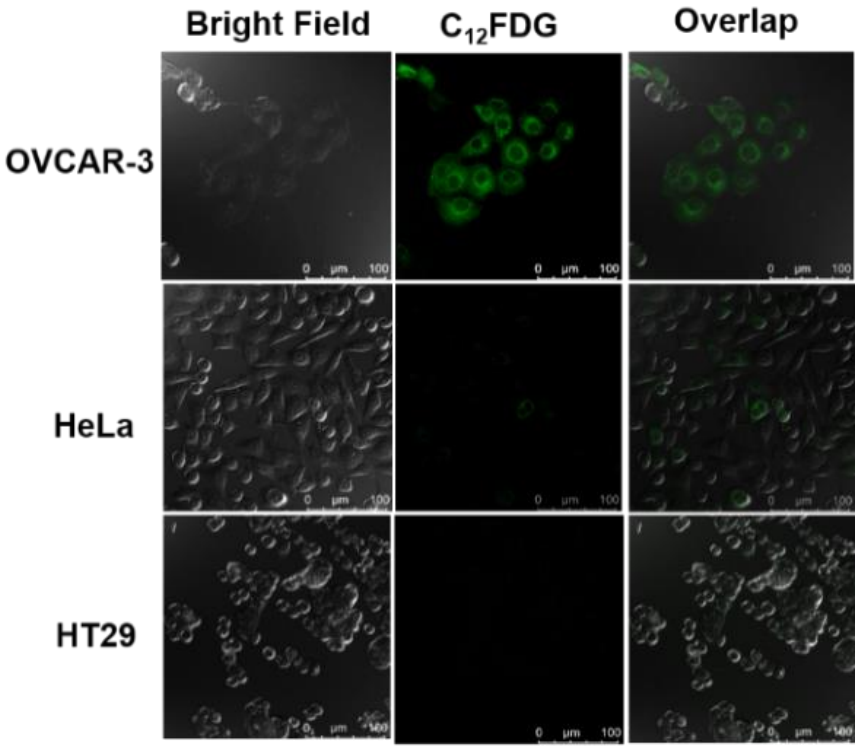


Fig. S4 Confocal fluorescence microscopic images of OVCAR-3, HeLa and HT29 cells after incubation with C_{12}FDG (25 μM) at 37 ℃ for 35 min.

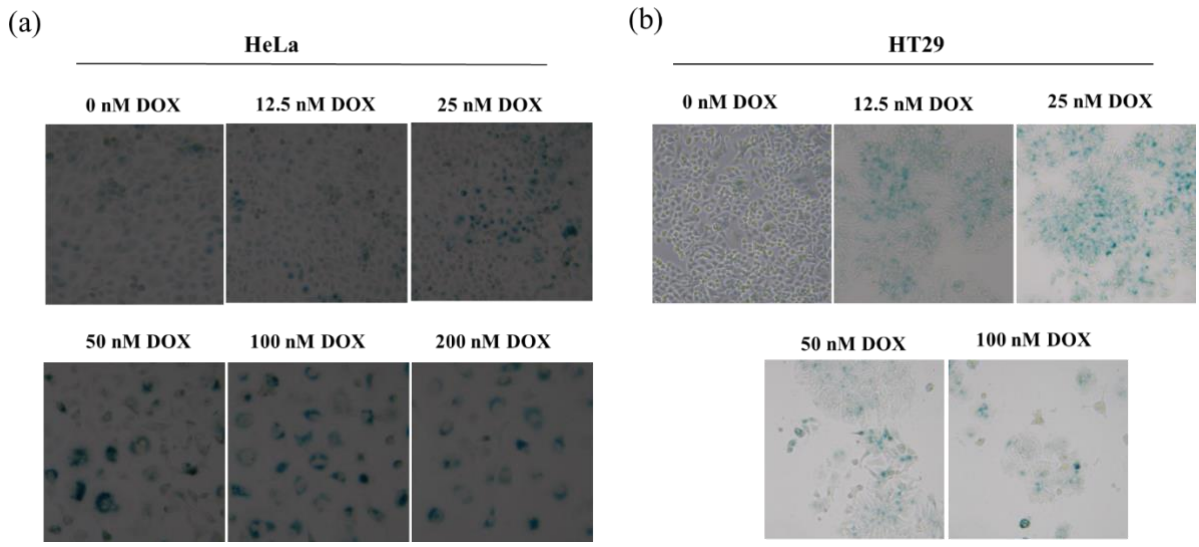


Fig. S5 Conventional X-Gal assay for assessing the β-gal levels in (a) HeLa and (b) HT29 cells after the treatment with various concentrations of DOX.

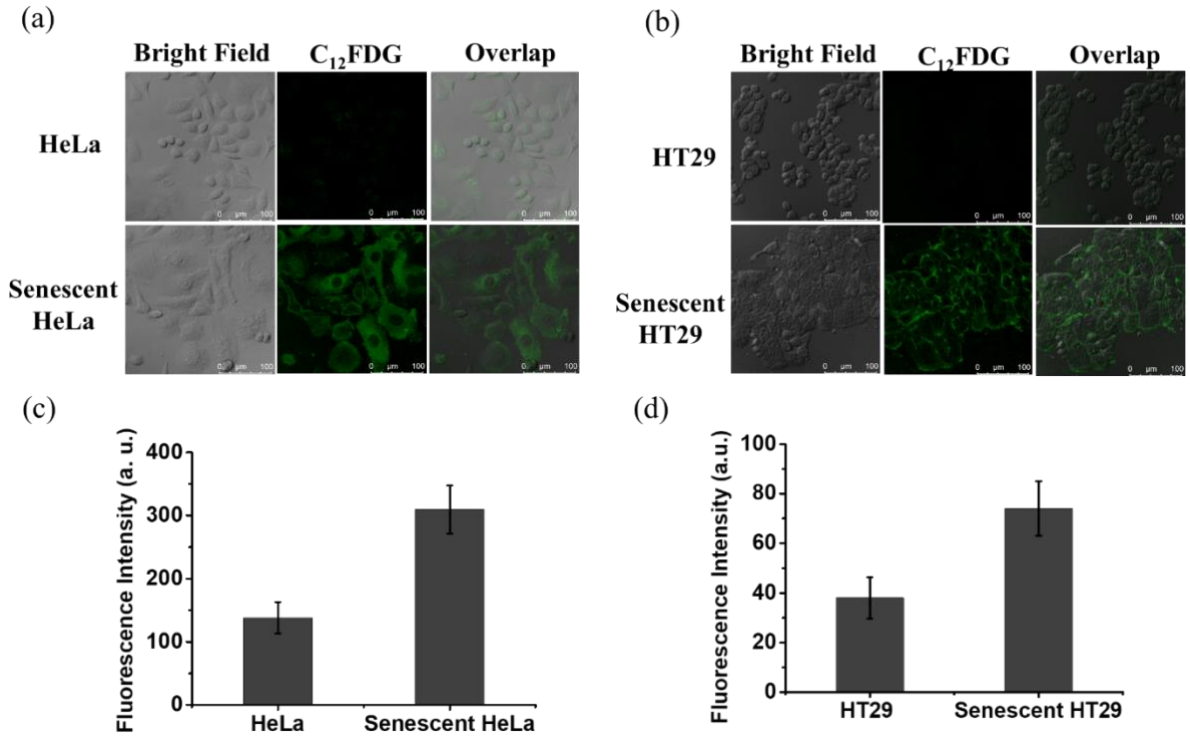
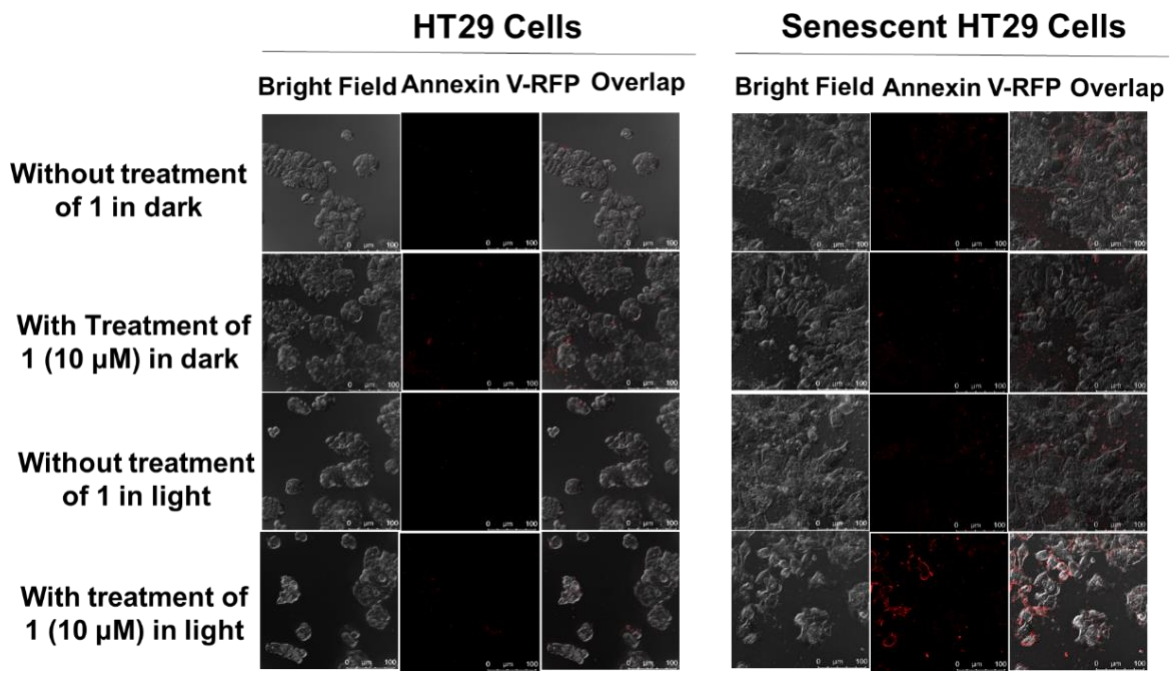


Fig. S6 Confocal microscopic images of (a) HeLa and senescent HeLa cells and (b) HT29 and senescent HT29 cells after incubation with C_{12}FDG (25 μM) for 35 min. The corresponding intracellular fluorescence intensities as determined by flow cytometry are given in figures (c) and (d) respectively. Data are expressed as the mean ± standard deviation (SD) of three independent experiments.

(a)



(b)

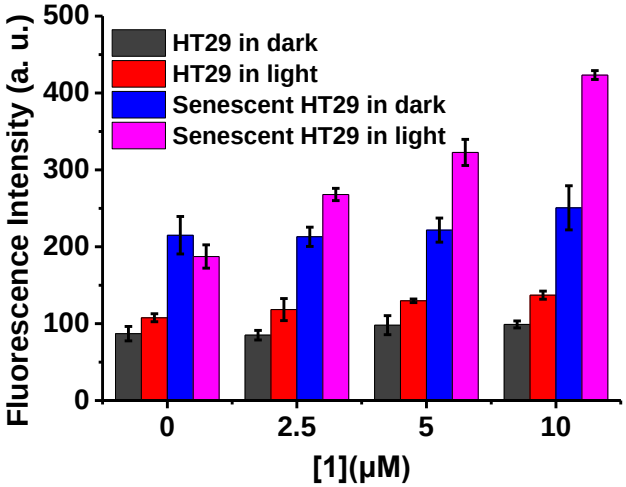


Fig. S7 (a) Confocal microscopic images of HT29 and senescent HT29 cells with or without the treatment with **1** (10 μM) for 2 h and light irradiation (λ > 515 nm, 25.5 mW cm^{-2}) for 20 min, followed by staining with annexin V-RFP for 15 min. (b) Comparison of the corresponding fluorescence intensities determined by flow cytometry, using different concentrations of **1**. Data are expressed as the mean ± SD of three independent experiments.

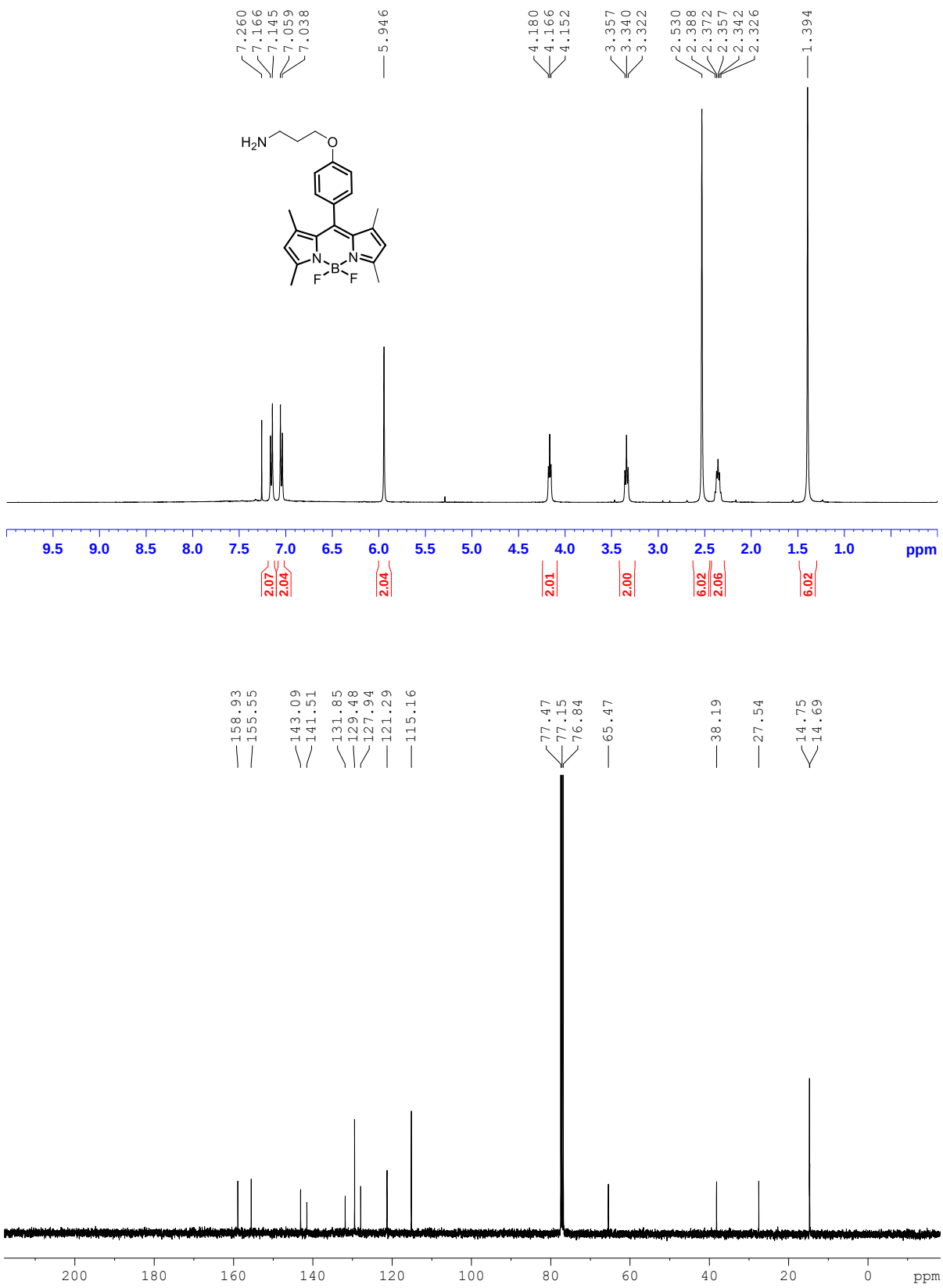


Fig. S8 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **4** in $CDCl_3$.

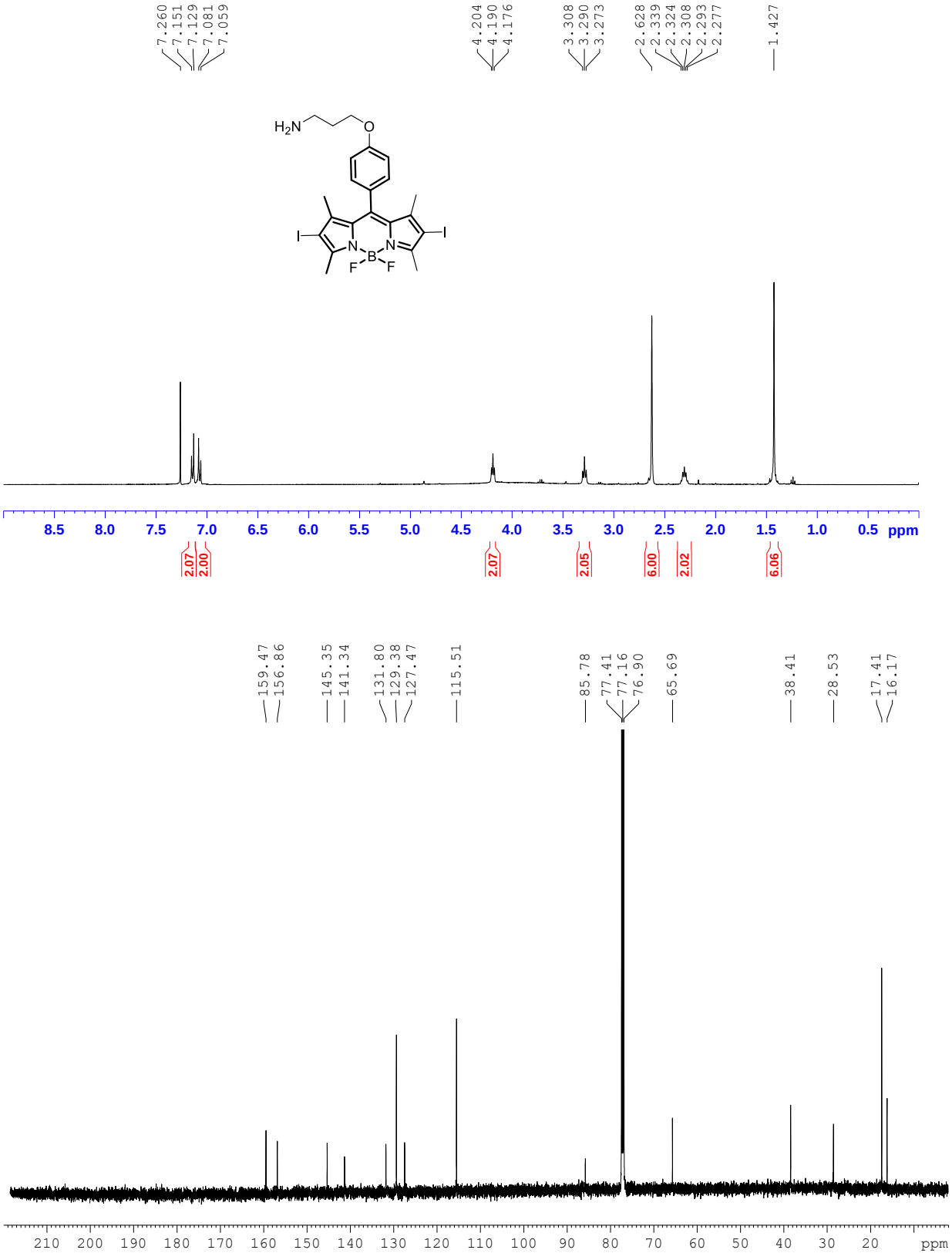


Fig. S9 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **2** in $CDCl_3$.

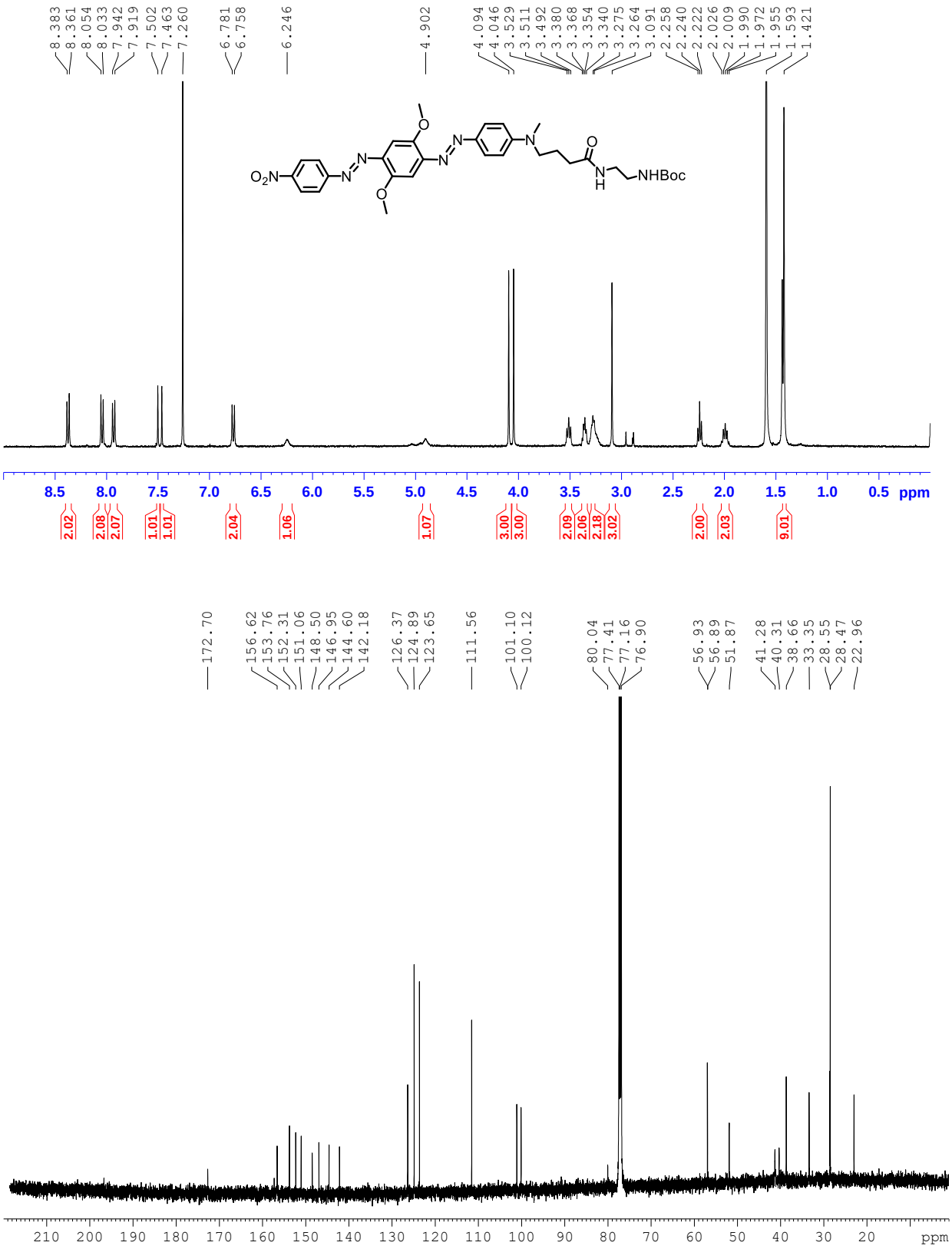


Fig. S10 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **7** in $CDCl_3$.

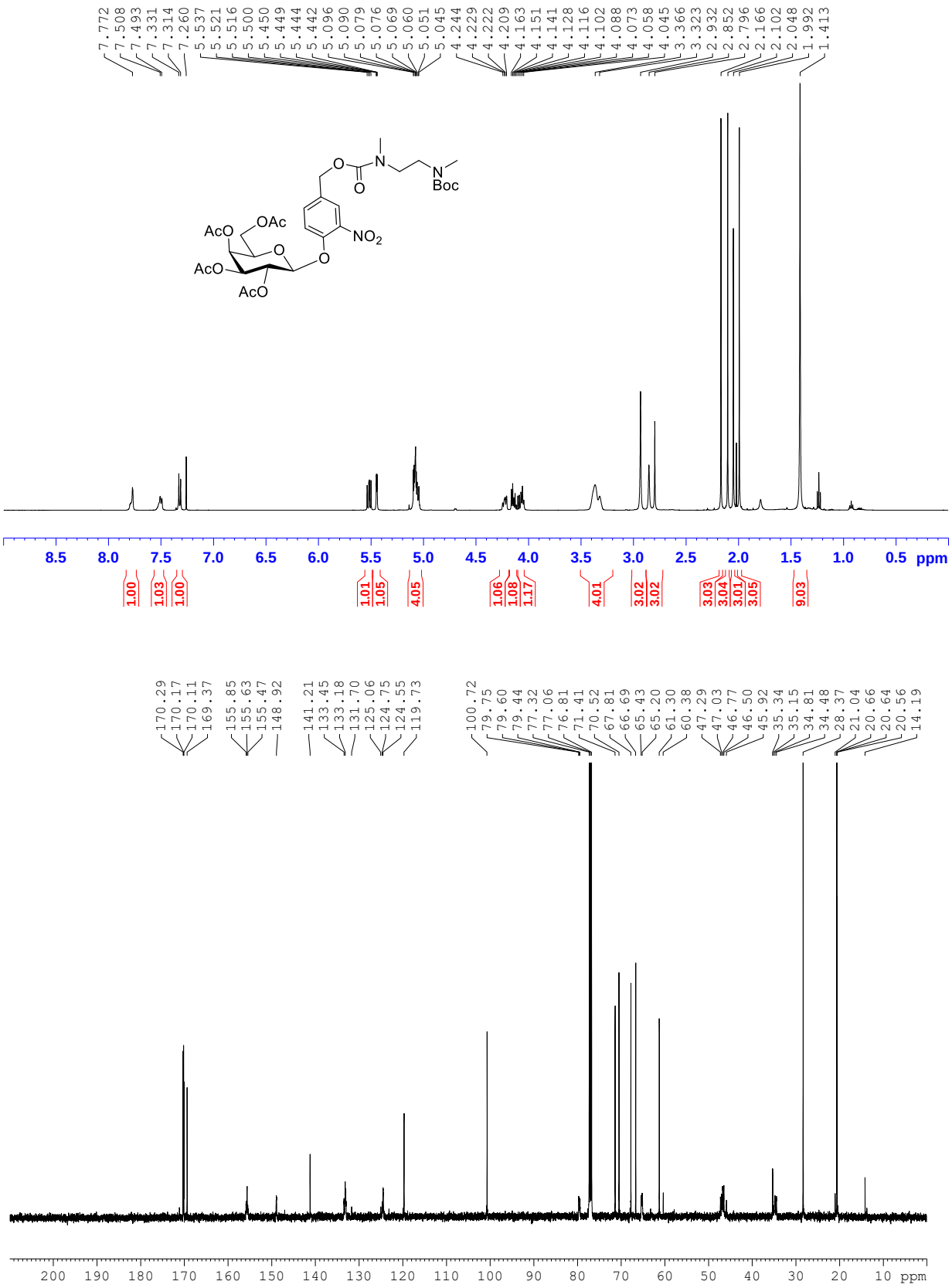


Fig. S11 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **11** in $CDCl_3$.

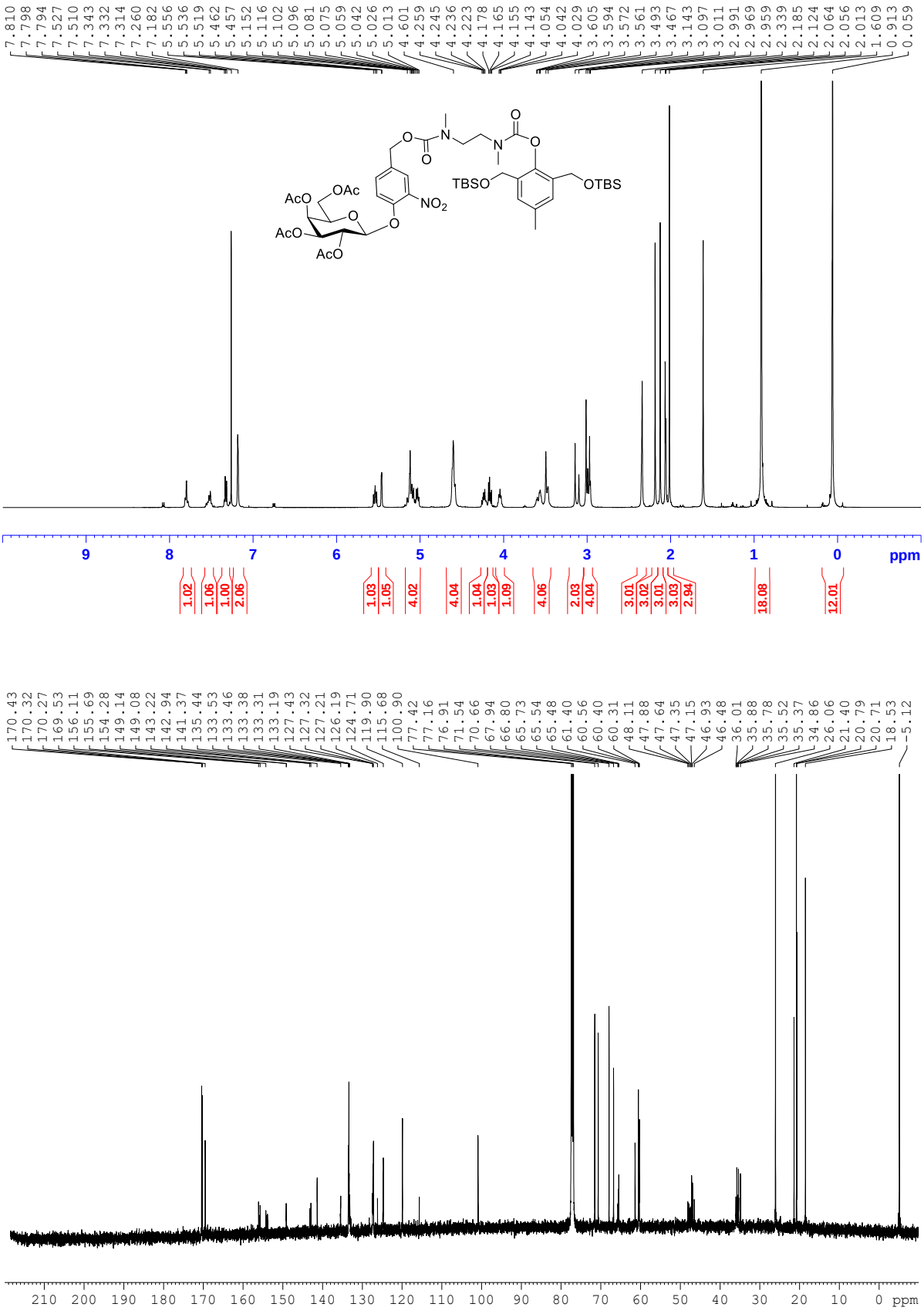


Fig. S12 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **14** in $CDCl_3$.

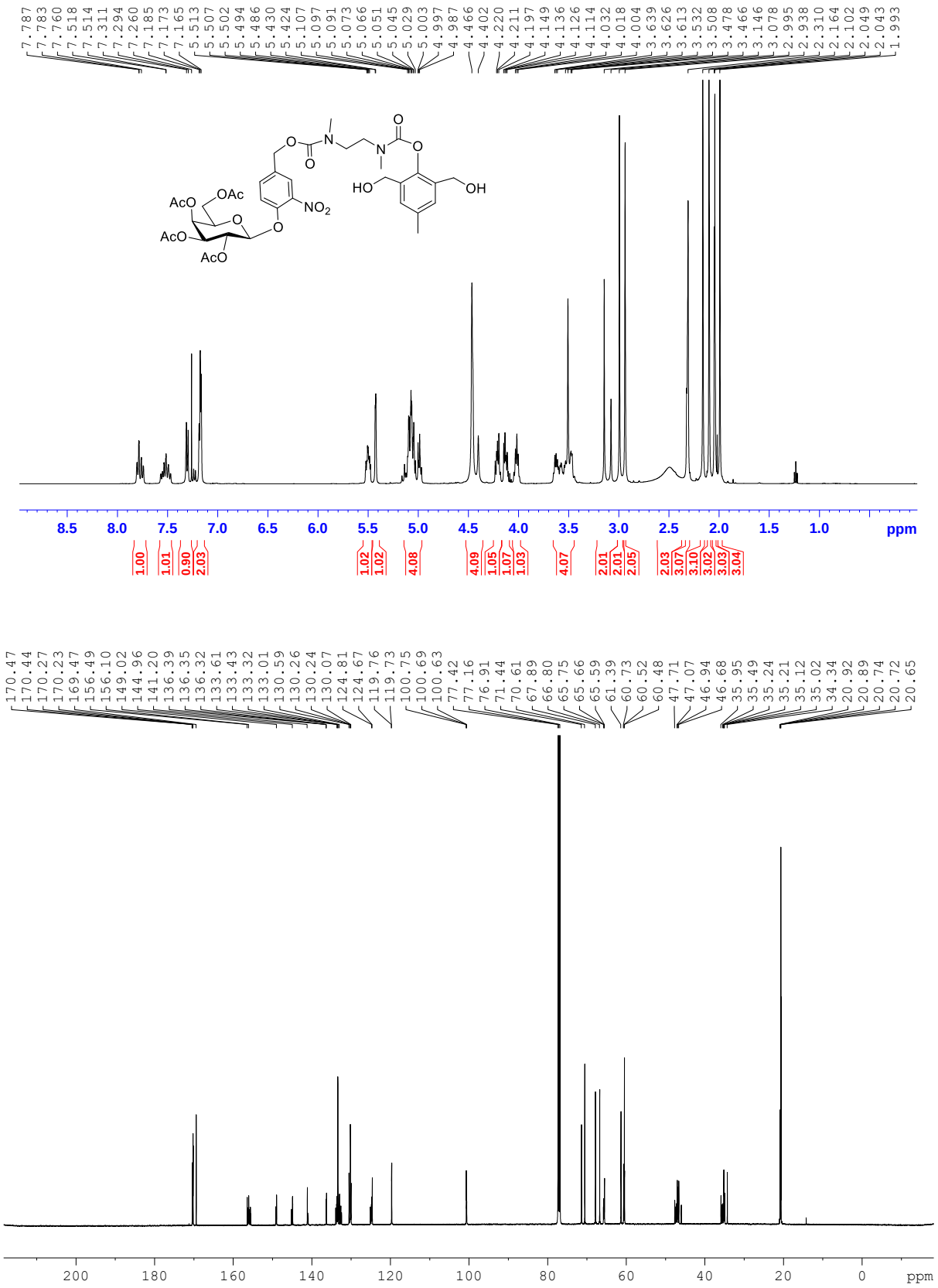


Fig. S13 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **15** in $CDCl_3$.

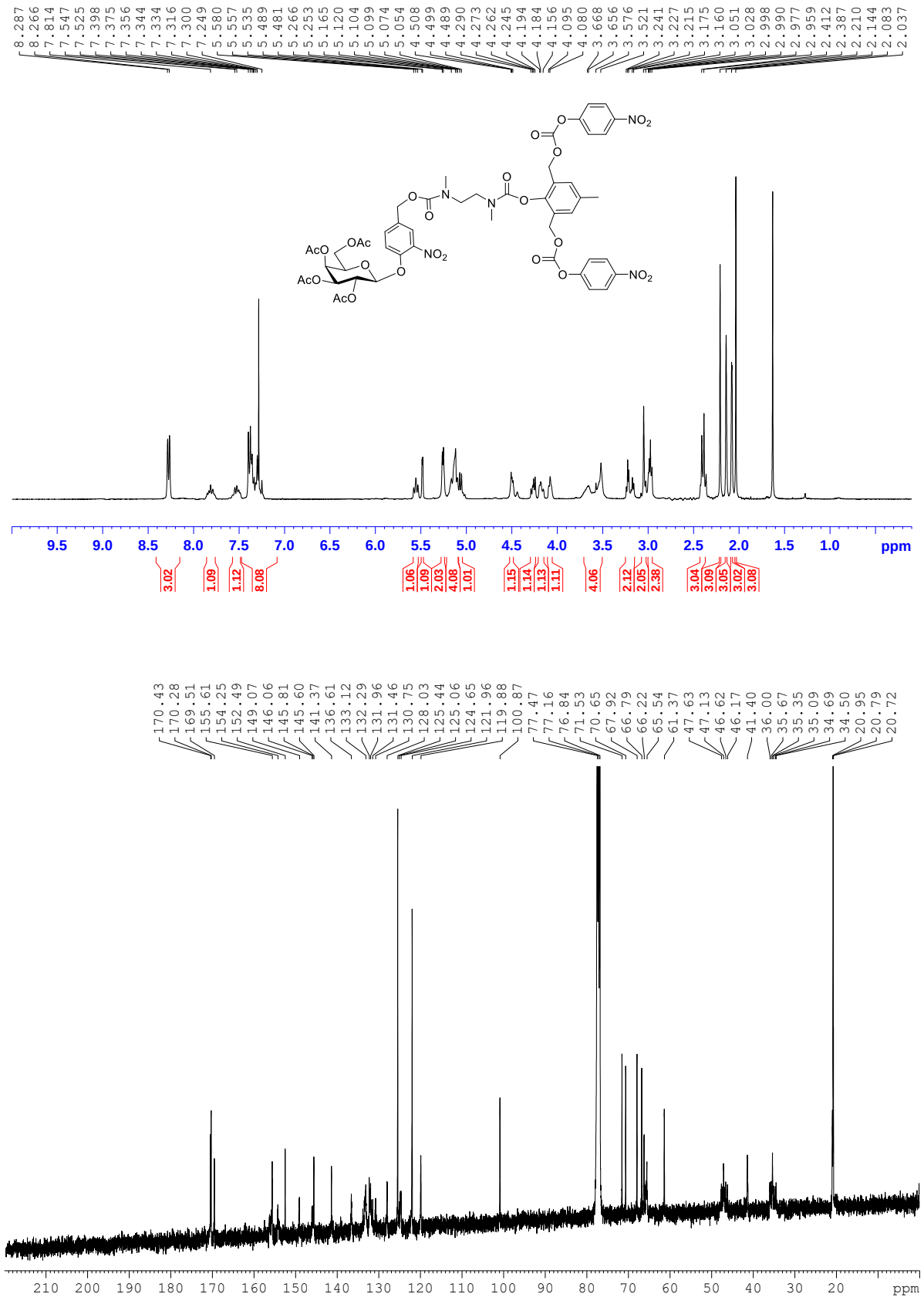


Fig. S14 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **17** in $CDCl_3$.

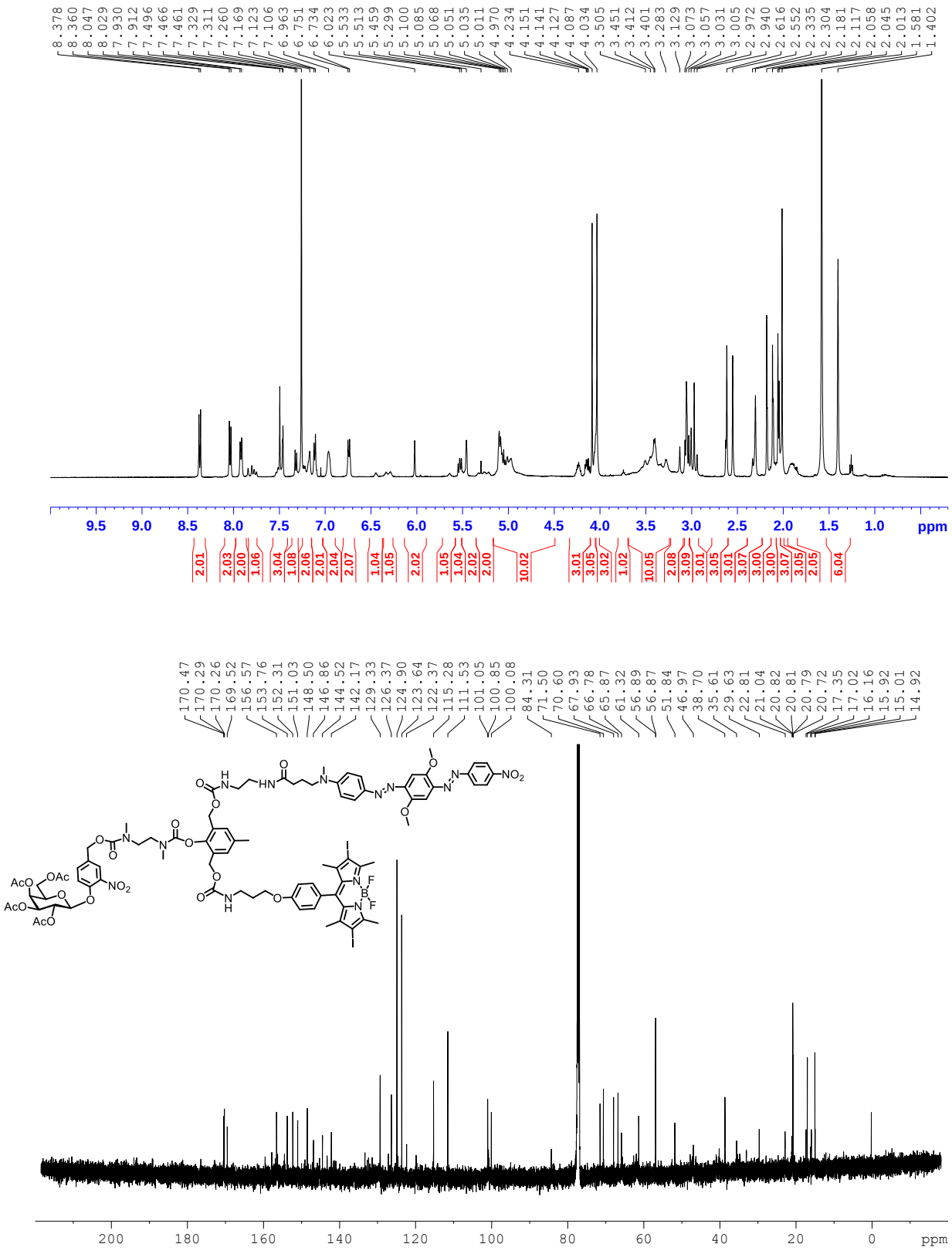


Fig. S15 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **18** in $CDCl_3$.

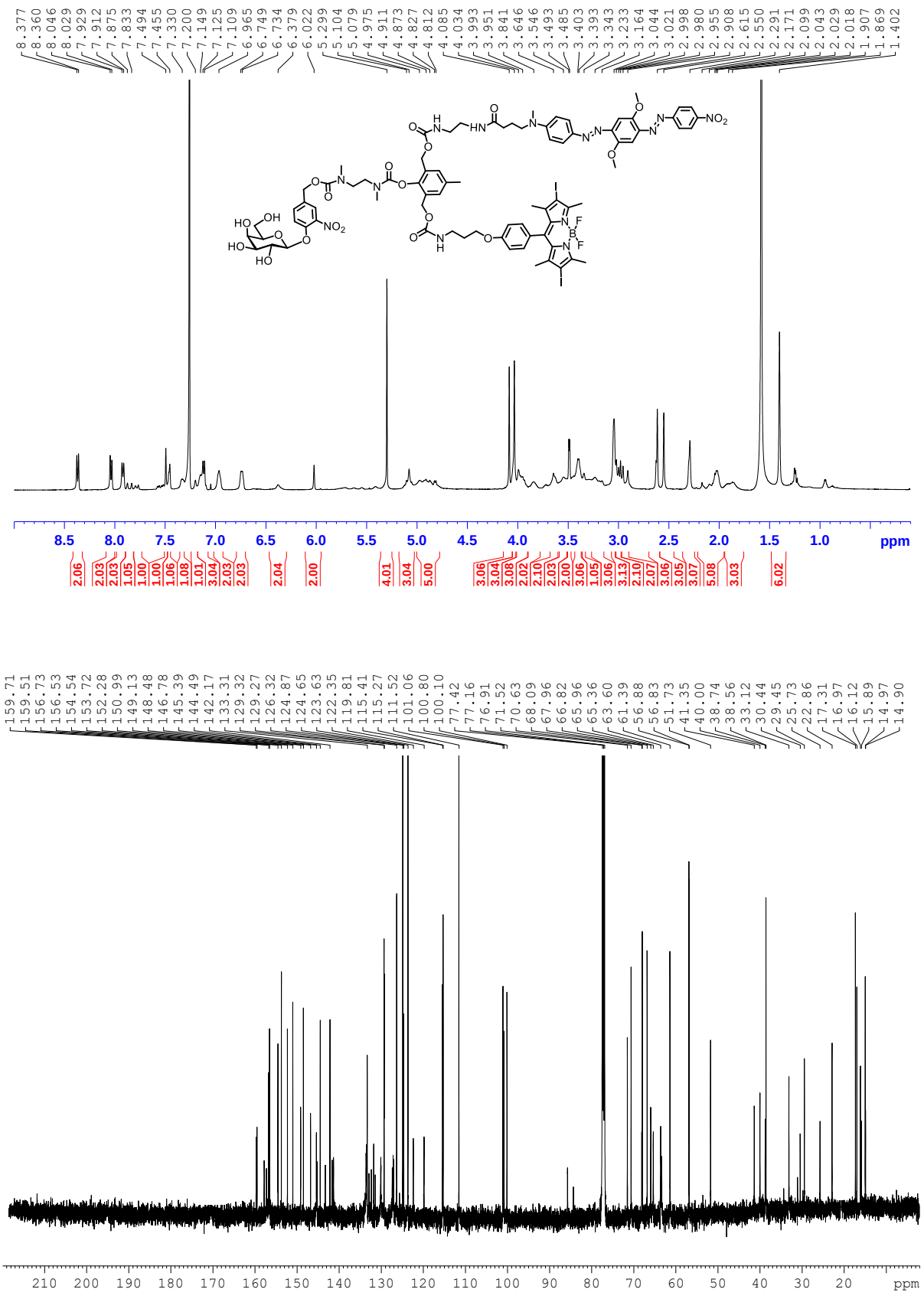


Fig. S16 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **1** in $CDCl_3$.

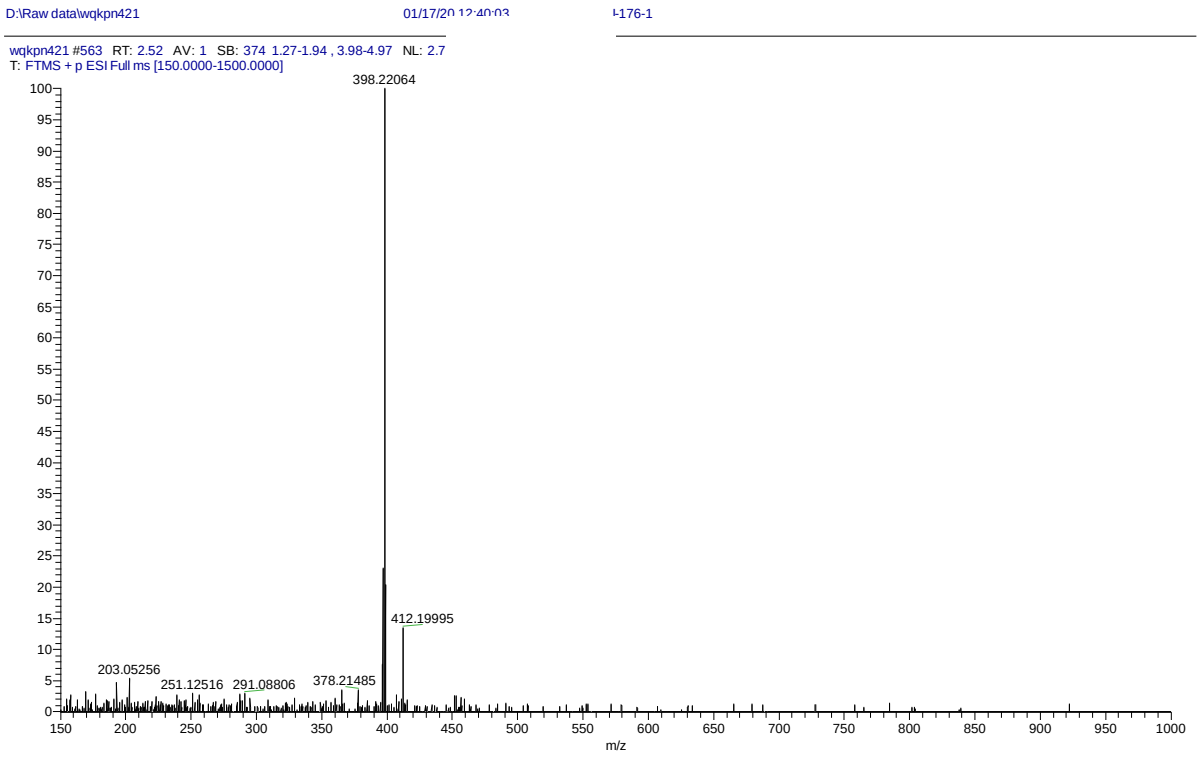


Fig. S17 ESI mass spectrum of **4**.

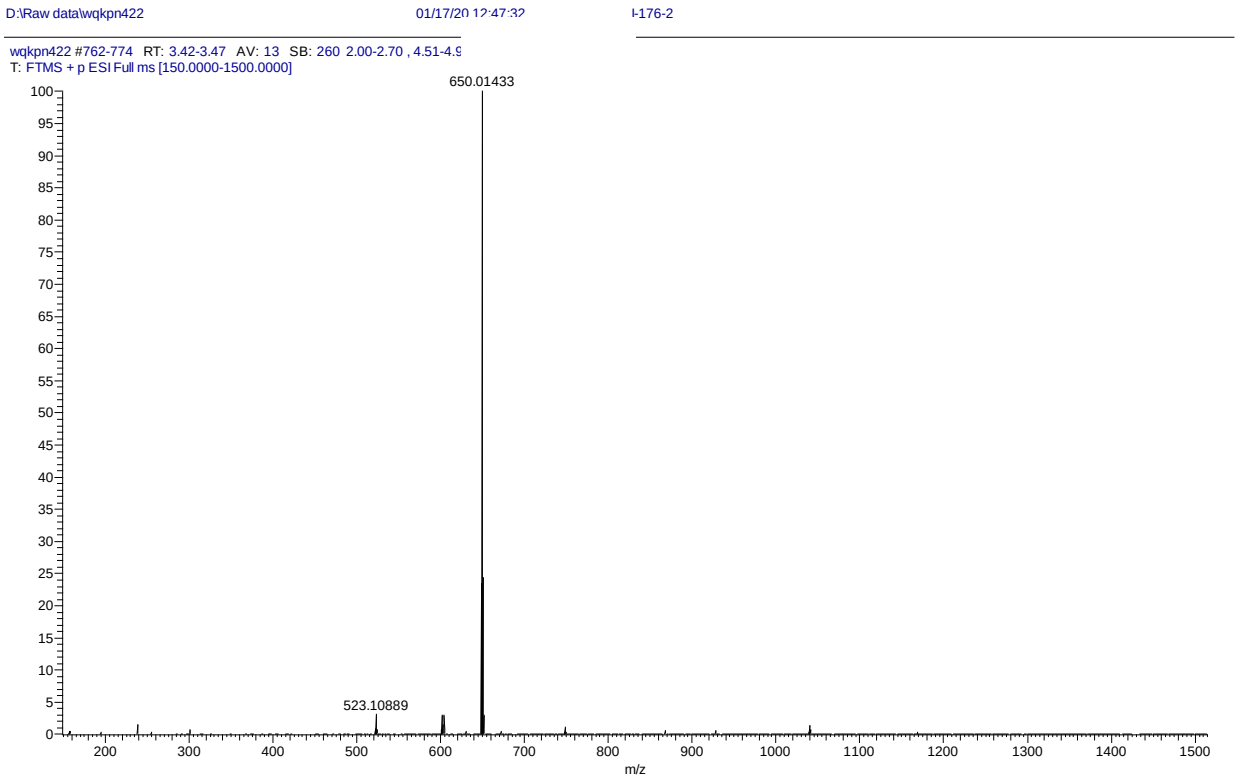


Fig. S18 ESI mass spectrum of **2**.

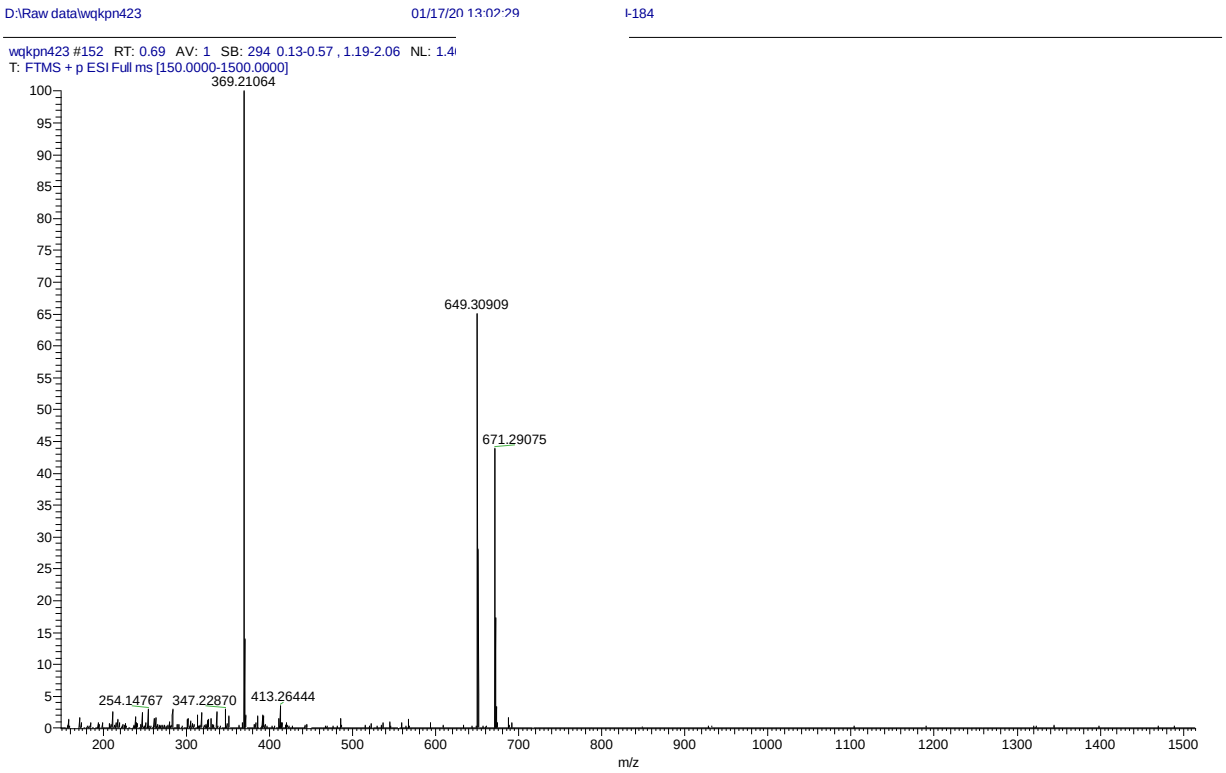


Fig. S19 ESI mass spectrum of **7**.

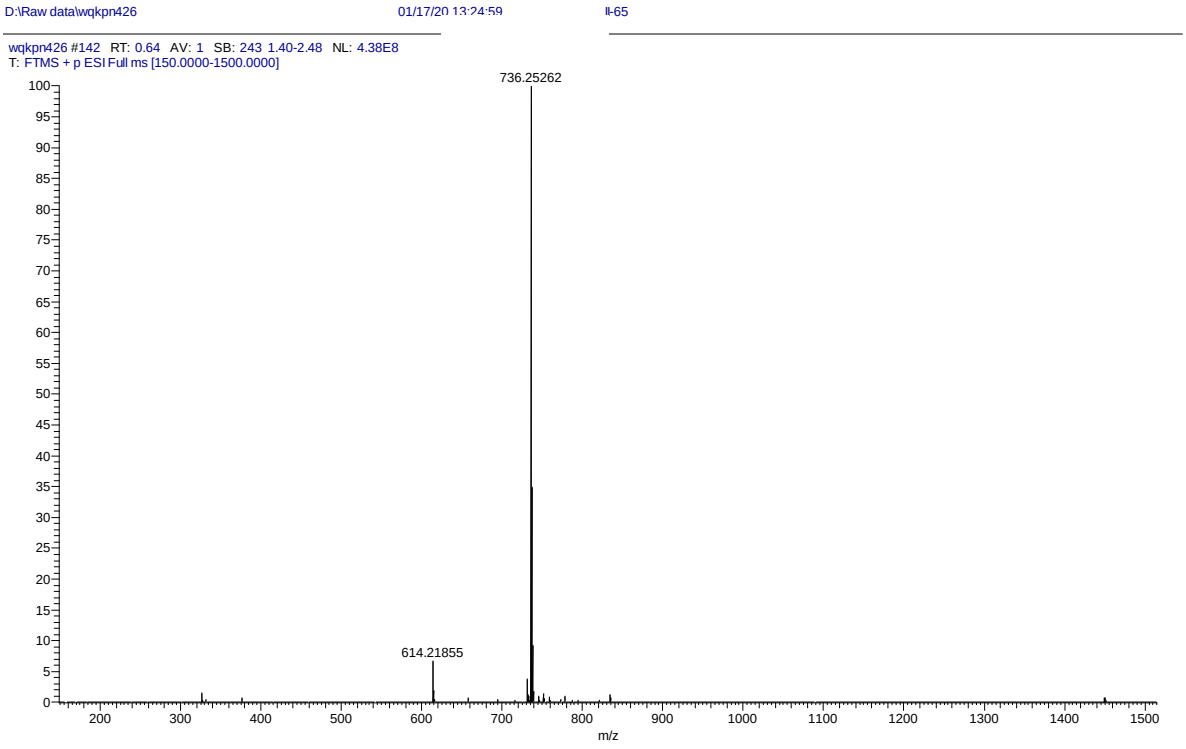


Fig. S20 ESI mass spectrum of **11**.

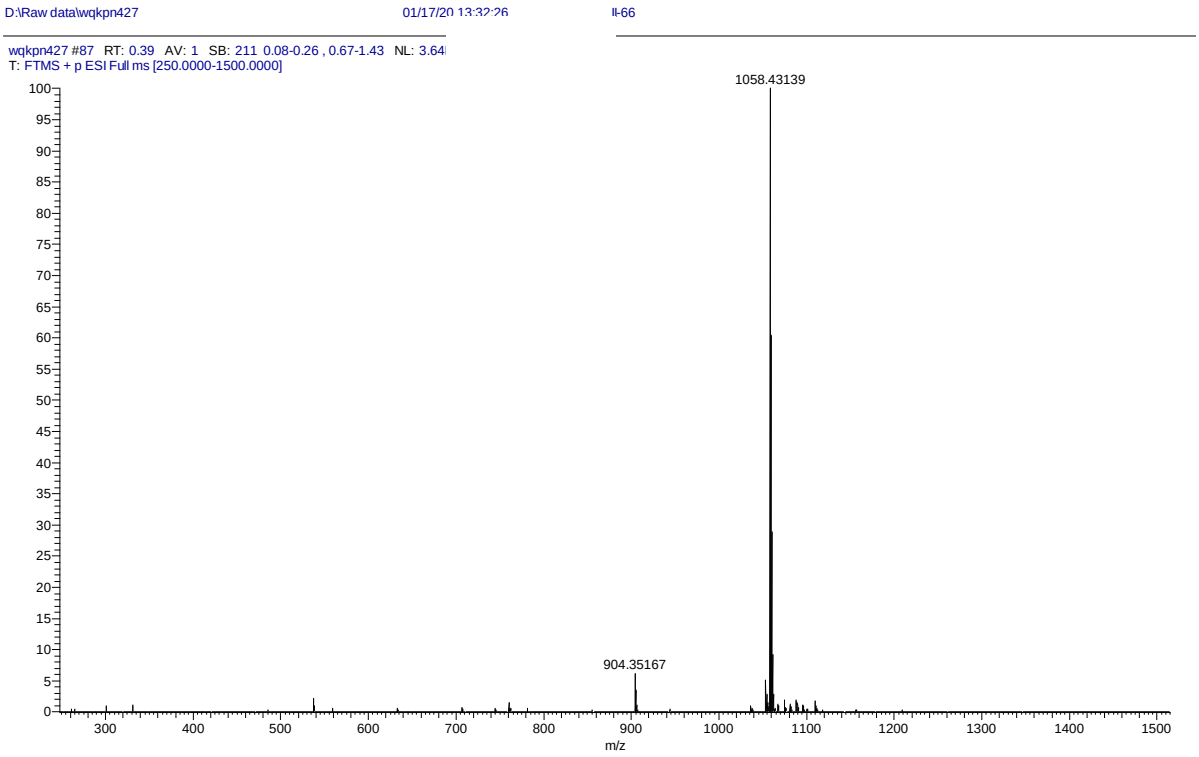


Fig. S21 ESI mass spectrum of **14**.

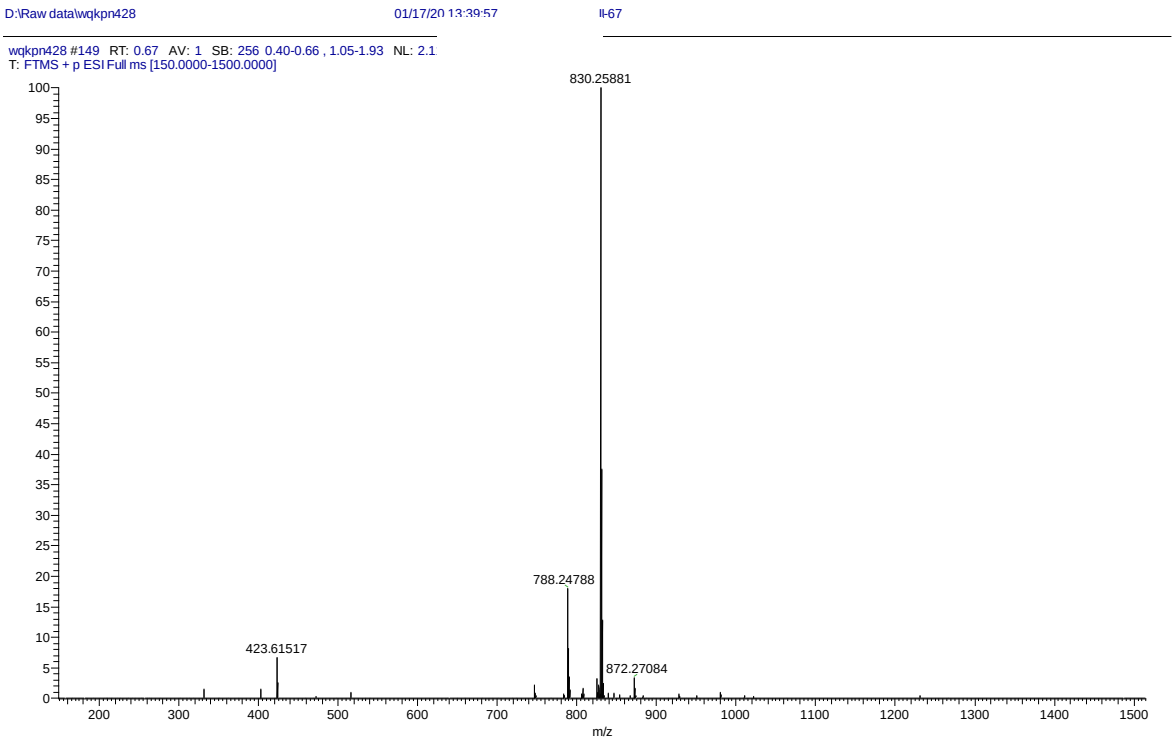


Fig. S22 ESI mass spectrum of **15**.

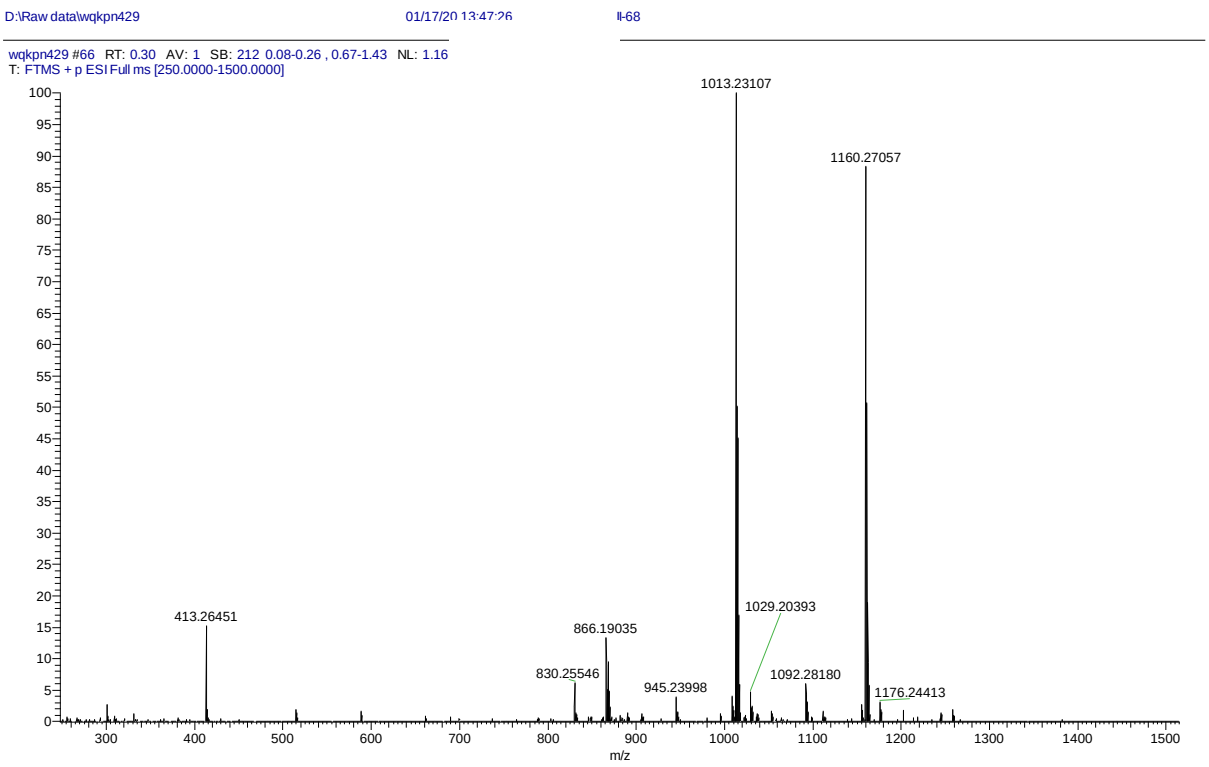


Fig. S23 ESI mass spectrum of **17**.

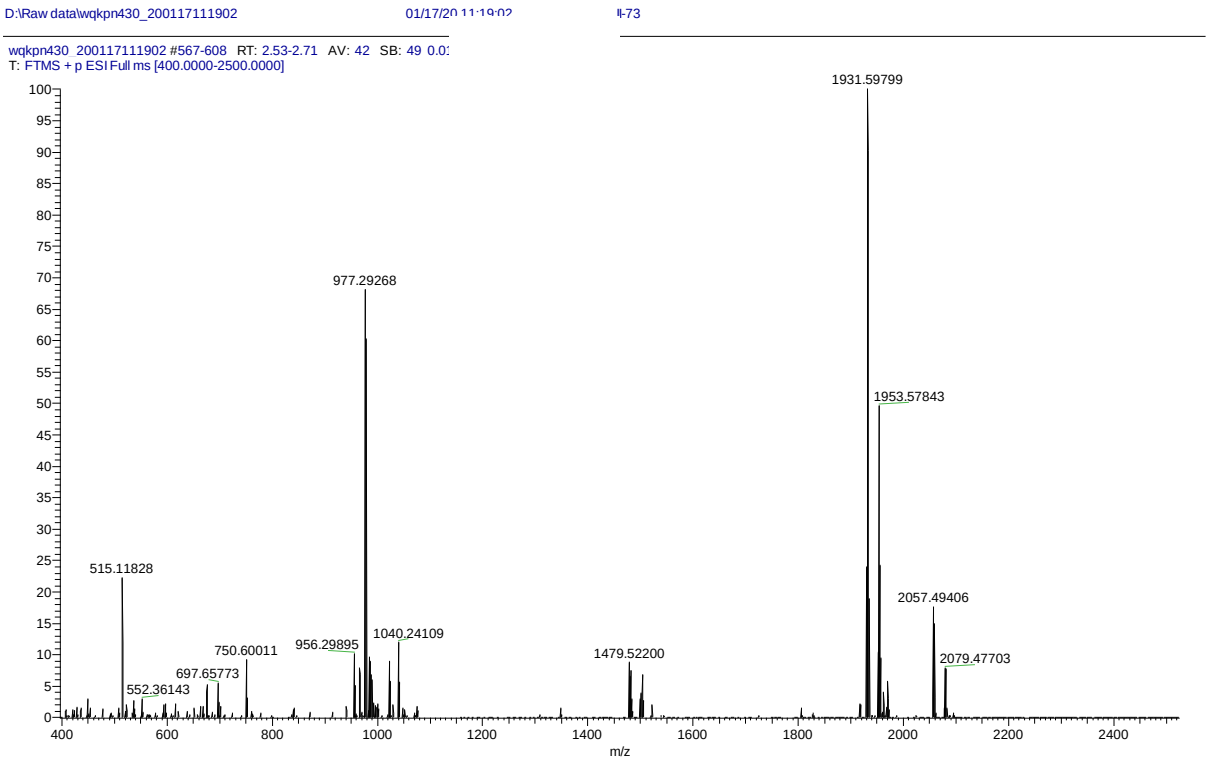


Fig. S24 ESI mass spectrum of **18**.

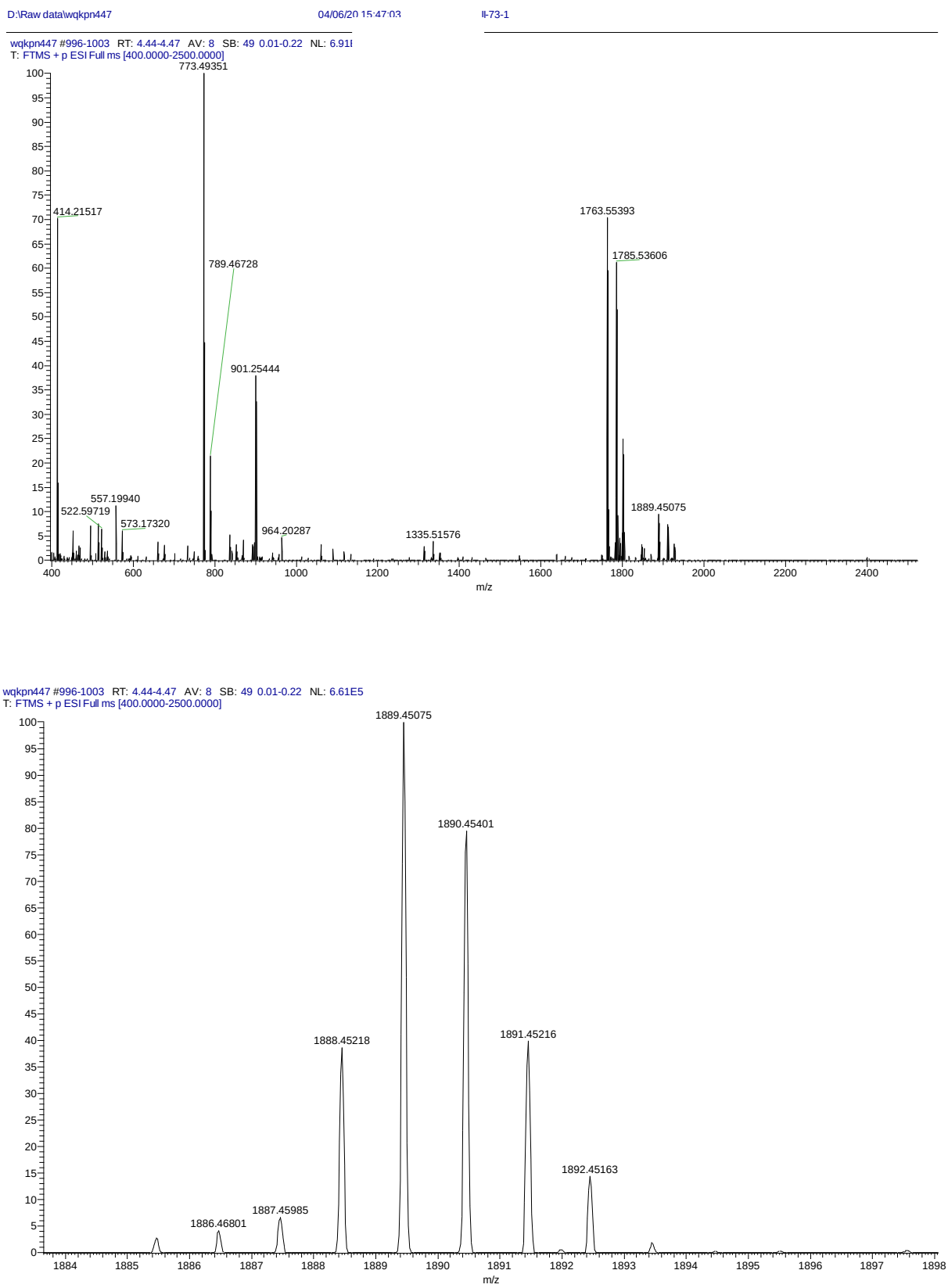


Fig. S25 ESI mass spectrum of **1**. An enlarged isotopic envelope for the $[M+H]^+$ species is shown on the bottom.