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Supplementary Information (ESI)

for

Enhancing Cellular Sulfane Sulfur Through a β-glycosidase-Activated Persulfide Donor: Mechanistic Insights and Oxidative Stress Mitigation

Prerona Bora,[a] Manjima B. Sathian[a] and Harinath Chakrapani *[a]

[a]Department of Chemistry, Indian Institute of Science Education and Research Pune, Dr. Homi Bhabha Road, Pashan Pune 411008, Maharashtra, India. E-mail: harinath@iiserpune.ac.in

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General methods

All reactions were conducted under nitrogen atmosphere. All the chemicals and solvents were purchased from commercial sources and used as received unless stated otherwise. Column chromatography was performed using silica gel-Rankem (60– 120 mesh) or silica gel Spectrochem (100-200 mesh) as the stationary phase. Preparative high-performance liquid chromatography (HPLC) was done using Combiflash EZ prep UV using a Kromasil®C-18 preparative column (250 mm × 21.2 mm, 5 µm). ^{1}H and ^{13}C spectra were recorded on a JEOL 400 MHz (or 100 MHz for ^{13}C) or a Bruker 400 MHz (or 100 MHz for ^{13}C) spectrometer unless otherwise specified using either residual solvent signals ($CDCl_3$ ™H= 7.26 ppm, ™C = 77.2 ppm) or as an internal tetramethylsilane (™H = 0.00, ™C = 0.0). Chemical shifts (™) are reported in ppm and coupling constants (*J*) in Hz. The following abbreviations are used: bs (broad signal), m (multiplet), s (singlet), d (doublet), t (triplet) and dd (doublet of doublets). High-resolution mass spectra were obtained from HRMS-ESI-Q-Time of Flight LC/MS. FT-IR spectra were recorded using BRUKER-ALPHA FT-IR spectrometer. Analytical HPLC was performed on an Agilent1260-infinity with Phenomenex®C-18 reverse phase column (250 mm × 4.6 mm, 5 µm). Photometric measurements were performed using a Thermo Scientific Varioskan microtiter plate reader.

Synthesis and Characterization:

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-bromotetrahydro-2*H*-pyran-3,4,5-triyl triacetate (4):

To a solution of β-D-glucose pentaacetate (1.5 g, 3.84 mmol) in anhydrous DCM (10 mL), HBr in acetic acid (6.22 mL, 76.86 mmol) was added dropwise at 0 °C under N_2 atmosphere and stirred at room temperature for 2 h. After completion of the reaction (TLC analysis), the reaction mixture was quenched with $NaHCO_3$ followed by extraction with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to afford the crude product. The product was immediately used in the next step without further purification.

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-nitrophenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (5)[1]:

To a solution of 2-nitrophenol (507 mg, 3.65 mmol) in anhydrous ACN (8 mL), Ag_2O (845 mg, 3.65 mmol) was added. To this heterogenous reaction mixture, a solution of compound **4** in ACN

was added dropwise under N_2 atmosphere at 0 °C. The reaction mixture was gradually warmed up to room temperature and left to stir for 12 h. After completion of the reaction as determined by TLC, the reaction mixture was filtered through celite and washed with $CHCl_3$. The filtrate was evaporated, the crude product obtained was diluted with H_2O and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The crude obtained was washed with hexane multiple times to obtain compound **5** (1.46 g, 85%) as an off-white solid. FT-IR (ν_{max}; cm^{-1}): 1531, 1221; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.35 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.23 – 7.19 (m, 1H), 5.32 – 5.29 (m, 2H), 5.21 – 5.18 (m, 1H), 5.13 – 5.11 (m, 1H), 4.27 – 4.24 (m, 2H), 3.89 – 3.85 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.4, 169.5, 149.4, 141.7, 133.8, 125.3, 124.0, 120.1, 100.3, 72.4, 70.6, 68.3, 61.9, 20.8, 20.7, 20.6; HRMS (ESI) for $C_{20}H_{23}NO_{12}$ $[M+Na]^+$ Calculated: 492.1117, Found: 492.1116.

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-aminophenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (6):

Compound **5** (300 mg, 0.63 mmol) was dissolved in dry THF (5 mL) and degassed for 10 min. To this solution was added Zinc dust (232 mg, 3.2 mmol) following which 6N HCl (532.8 µL, 3.2 mmol) was added dropwise at 0 °C and the reaction was stirred at room temperature for 36 h. Upon completion of the reaction (TLC analysis), the reaction mixture was passed through celite and washed with ethyl acetate. The filtrate was treated with 1N $NaHCO_3$ until the pH of the solution turned neutral. The mixture was then extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The crude product obtained was washed with diethyl ether (3 × 2 mL) to obtain compound **6** as a light brown solid (271.57 mg, 96%). FT-IR (ν_{max}; cm^{-1}): 2922, 1621, 1372; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 – 6.88 (m, 2H), 6.70 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.68 – 6.64 (m, 1H), 5.31 – 5.29 (m, 2H), 5.18 – 5.13 (m, 1H), 4.99 – 4.97 (m,1H), 4.30 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.18 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.86 – 3.82 (m, 1H), 3.79 (s, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.3, 169.9, 169.6, 144.5, 137.7, 124.4, 118.3, 116.3, 115.9, 100.6, 72.6, 72.2, 71.4, 68.5, 62.0, 20.9, 20.8, 20.8, 20.7; HRMS (ESI) for $C_{20}H_{25}NO_{10}$ $[M+H]^+$ Calculated: 440.1556, Found: 440.1556.

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-(methylamino)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (7)[2]: Formic acid (257.56 µL, 6.83 mmol) and acetic anhydride (645.36 µL, 6.83 mmol) was heated at 70 °C for 2 h. The reaction mixture was cooled to room temperature and to that a solution of compound **6** (300 mg, 0.68 mmol) in THF (5 mL) was added. The reaction was stirred for 3 h at room temperature. Following complete consumption of starting material (TLC analysis), the reaction mixture was poured into hexane (20 mL) and the mixture was stirred at for 30 min. The precipitate obtained was filtered, washed with hexane and dried to obtain the formyl product.

To a solution of the above formyl compound in dry THF (5 mL), 2M borane dimethyl sulfide (975 µL) was added dropwise at 0 °C under N_2 atmosphere. The reaction was stirred for 4 h at room temperature. Upon completion as monitored by TLC, the reaction was quenched using methanol and the solvent was evaporated to obtain the crude product. The product was then suspended in water and extracted using EtOAc (3 × 20 mL), washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated. The product was washed with diethyl ether (3 × 2 mL) to obtain compound **7** as a pale brown solid (276.45 mg, 75%). FT-IR (ν_{max}; cm^{-1}): 1607, 1518, 1220; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 – 6.98 (td, *J* = 7.6, 1.2 Hz, 1H), 6.90 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.61 – 6.59 (m, 2H), 5.31 – 5.28 (m, 2H), 5.18 – 5.13 (m, 1H), 4.98 – 4.96 (m, 1H), 4.30 (dd, *J* = 12.4, 5.2 Hz, 1H), 4.17 (dd, *J* = 12.4, 2.4 Hz, 1H), 3.86 – 3.82 (m, 1H), 2.82 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.3, 170.0, 169.6, 144.2, 140.6, 124.6, 116.0, 115.2, 110.3, 100.6, 72.6, 72.1, 71.4, 68.5, 62.0, 30.3, 20.9, 20.9, 20.8, 20.7; HRMS (ESI) for $C_{21}H_{27}NO_{10}$ $[M+H]^+$ Calculated: 454.1713, Found: 454.1715.

Methyl *N*-acetyl-S-((chlorocarbonyl)thio)-L-cysteinate (8a):

To a solution of compound *N*-acetylcysteine methyl ester (65 mg, 0.35 mmol) in anhydrous $CHCl_3$ (4 mL), chlorocarbonyl sulfenyl chloride (62 µL, 0.7 mmol) was added dropwise at 0 °C. The reaction was complete in 30 min as indicated by TLC. This intermediate was immediately taken forward without further purification.

Benzyl carbonochlorido(dithioperoxoate) (8b)

To a solution of benzyl mercaptan (50 mg, 0.4 mmol) in anhydrous $CHCl_3$ (4 mL), chlorocarbonyl sulfenyl chloride (68 µL, 0.8 mmol) was added dropwise at 0 °C. The reaction was complete within

45 min as indicated by TLC. This intermediate was immediately taken forward without further purification.

General procedure for synthesis of compounds 9a and 9b[3]: Compound **7** was dissolved in anhydrous $CHCl_3$ and DIPEA (5 equivalents) was added dropwise at 0 °C. This was followed by the addition of compound **8** (2 equivalents) to the reaction mixture and the reaction was stirred at room temperature. Upon completion (TLC analysis), the reaction was quenched by adding water and the product was extracted using $CHCl_3$ (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated. The crude obtained was purified by reverse phase HPLC using ACN-H_2O as the eluent.

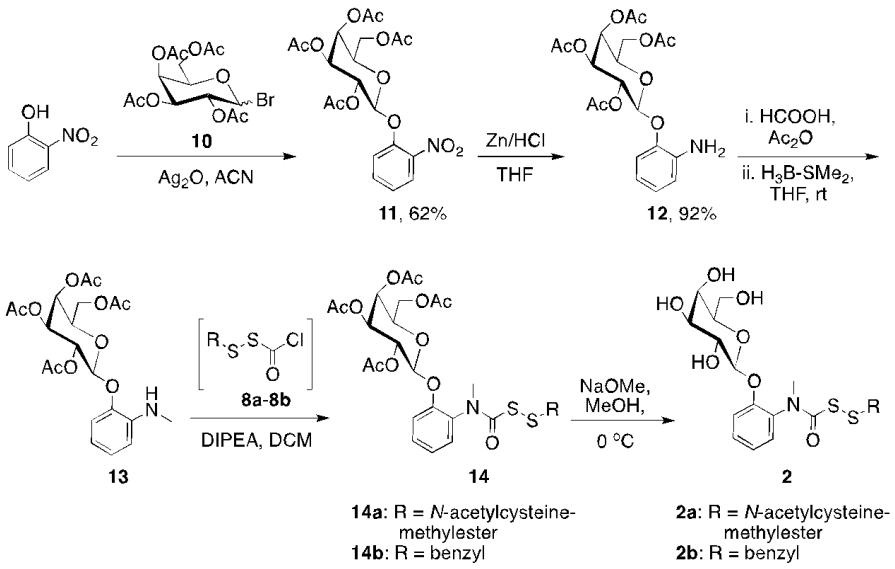
(2*S*,3*R*,4*S*,5*R*,6*R*)-2-(2-(((2-acetamido-3-methoxy-3-oxopropyl)disulfannecarbonyl) (methyl)amino)phenoxy)-6-(acetoxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (9a):

Following the general procedure outlined above, compound **7** (80 mg, 0.18 mmol) was reacted with **8a** in the presence of DIPEA (160 μL, 0.9 mmol) and the reaction was stirred for 3 h. Compound **9a** was obtained after purification (10 mg, 23%). FT-IR (ν_{max}; cm^{-1}): 1678, 1500; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 46.8, 7.2 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.29 – 7.28 (m, 1H), 7.14 – 7.10 (m, 1H), 5.29 – 5.09 (m, 4H), 4.83 – 4.78 (m, 1H), 4.27 – 4.20 (m, 2H), 3.93 – 3.9 (m, 1H), 3.73 (s, 1H), 3.64 – 3.39 (m, 1H), 3.27 (s, 1H), 3.02 (ddd, *J* = 29.2, 14.4, 5.2 Hz, 1H), 2.11 – 2.03 (m, 15 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.7, 170.6, 170.3, 169.6, 166.7, 153.9, 131.5, 131.4, 130.2, 124.3, 117.7, 99.4, 72.5, 72.3, 70.8, 68.4, 62.1, 52.7, 51.1, 38.4, 32.1, 23.2, 20.9, 20.8; HRMS (ESI) for $C_{28}H_{36}N_2O_{14}S_2$ $[M+H]^+$ Calculated: 689.1686, Found: 689.1686.

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-((benzyldisulfannecarbonyl)(methyl)amino) phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (9b):

Following the general procedure outlined above, compound **7** (90 mg, 0.2 mmol) was reacted with **8b** in the presence of DIPEA (165 μL, 1 mmol) and the reaction was stirred for 4 h. Compound **9b** was obtained as a white solid after purification (12 mg, 25%). FT-IR (ν_{max}; cm^{-1}): 1683, 1497; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.29 (m, 7H), 7.22 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.11 (td, *J* = 7.2, 1.6 Hz, 1H), 5.32 – 5.24 (m, 2H), 5.18 – 5.13 (m, 1H), 5.04 (d, *J* = 7.2 Hz, 1H), 4.29 – 4.23 (m, 2H), 4.00 – 3.90 (m, 2H), 3.86 – 3.82 (m, 1H), 3.25 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.3, 169.7, 169.6, 166.1, 153.9, 136.5, 131.5,

131.2, 130.7, 129.7, 128.7, 127.8, 124.3, 118.2, 99.6, 72.6, 72.3, 70.7, 68.5, 62.1, 43.7, 38.0, 20.9, 20.8; HRMS (ESI) for $C_{29}H_{33}NO_{11}S_2$ $[M+H]^+$ Calculated: 636.1573, Found: 636.1583.



Scheme S1: Synthesis of the galactopyranosyl derivative, **2**.

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-bromotetrahydro-2*H*-pyran-3,4,5-triyl triacetate (10): Using a similar protocol as outlined for **5**, β-D-galactose pentaacetate (1.4 g, 3.6 mmol) was reacted with HBr in acetic acid (5.8 mL, 71.7 mmol) in anhydrous DCM. Compound **6** obtained was used immediately in the next step without further purification.

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-nitrophenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (11): Using a similar protocol as outlined for **5**, 2-nitrophenol (500 mg, 3.65 mmol) was reacted with **10** (1.5 g, 3.65 mmol) and Ag_2O (845 mg, 3.65 mmol) in anhydrous ACN. After completion of the reaction (TLC analysis), the reaction mixture was filtered by passing through celite and washed with $CHCl_3$ subsequently. The crude product obtained after evaporation of the filtrate was purified by column chromatography using 60-120 silica gel as the stationary phase. A gradient starting from 2% ethylacetate in hexane was used as the mobile phase and the compound was eluted at 50%. The product was obtained as an off-white solid (1.05 g, 62 %). FT-IR (ν_{max}; cm^{-1}): 1535, 1219; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.8 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.38 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.23 – 7.19 (m, 1H), 5.58 (dd, *J* = 12.0, 8.0 Hz, 1H), 5.48 (d, *J* = 4.0 Hz, 1H), 5.13 – 5.08 (m, 2H), 4.29 (dd, *J* = 12.0, 7.0 Hz, 1H), 4.19 (dd, *J* = 12.0, 7.0 Hz, 1H), 4.10 – 4.06 (m, 1H), 2.19 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (100

MHz, $CDCl_3$): δ 170.6, 170.4, 169.5, 149.4, 141.7, 133.8, 125.3, 124.0, 120.1, 100.3, 72.4, 70.6, 68.3, 61.9, 20.8, 20.7, 20.6; HRMS (ESI) for $C_{20}H_{23}NO_{12}$ $[M+Na]^+$ Calculated: 492.1117, Found: 492.1112.

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-aminophenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (12): Using a similar protocol as outlined for **6**, **11** (300 mg, 0.64 mmol) was reacted with Zn dust (418 mg, 6.4 mmol) and 6N HCl (530 µL, 3.2 mmol) in anhydrous THF (5 mL). The final product **12** was obtained as a light brown solid (260 mg, 92%). FT-IR (υ_{max}, cm^{-1}): 2926, 1618, 1371; ^1H NMR (400 MHz, $CDCl_3$): δ 6.98-6.88 (m, 2H), 6.72-6.65 (m, 2H), 5.53-5.46 (m, 2H), 5.14 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.98 (d, *J* = 8.0 Hz, 1H), 4.28 (m, 2H), 4.06 (t, *J* = 8.0 Hz, 1H), 3.8 (s, 2H), 2.19 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.3, 169.9, 169.6, 144.5, 137.7, 124.4, 118.2, 116.2, 115.9, 100.6, 72.6, 72.1, 71.3, 68.5, 62.0, 20.9, 20.8, 20.8, 20.7; HRMS for $C_{20}H_{25}NO_{10}$ $[M+K]^+$ Calculated: 478.1116, Found: 478.1110

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-(methylamino)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (13): Using a similar protocol as outlined for **7**, formic acid (215 µL, 5.7 mmol) and acetic anhydride (540 µL, 5.7 mmol) were reacted with **12** (250 mg, 0.57 mmol). This was followed by reduction with 2M borane dimethyl sulfide (850 µL) in anhydrous THF (5 mL). Upon completion of the reaction (TLC analysis), the reaction was quenched using methanol and the solvent was evaporated to obtain the crude product. The product was then suspended in water and extracted using EtOAc (3 × 20 mL), washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated to obtain compound **13**. The product **13** being unstable was immediately used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 7.02 – 6.98 (td, *J* = 7.6, 1.2 Hz, 1H), 6.90 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.62 – 6.59 (m, 2H), 5.51 – 5.46 (m, 2H), 5.13 (dd, *J* = 10.0, 4.0 Hz, 1H), 4.97 (d, *J* = 8.0 Hz, 1H), 4.26 – 4.14 (m, 3H), 4.07 (m, 1H), 2.83 (s, 3H), 2.19 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H).

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-((benzyldisulfannecarbonyl)(methyl) amino) phenoxy) tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (14b):

Compound **13** (90 mg, 0.2 mmol) was dissolved in anhydrous $CHCl_3$ and DIPEA (165 µL, 1 mmol) was added dropwise at 0 °C. This was followed by the addition of compound **8b** to the reaction mixture and the reaction was stirred at room temperature. Upon completion (TLC analysis), the

reaction was quenched by adding water and the product was extracted using $CHCl_3$ (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated. The crude obtained was purified by reverse phase HPLC using ACN-H_2O as the eluent. Compound **14b** was obtained as a white solid after purification (43 mg, 34%). FT-IR (ν_{max}; cm^{-1}): 1680, 1495; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.31 (m, 7H), 7.22 (dd, *J* = 12.0, 1.7 Hz, 1H), 7.14 (td, *J* = 8.0, 1.5 Hz, 1H), 5.54 – 5.45 (m, 2H), 5.09 (dd, *J* = 10.0, 4.0 Hz, 1H), 5.00 (d, *J* = 8.0 Hz, 1H), 4.29 – 4.25 (m, 1H), 4.19 – 4.15 (m, 1H), 4.05 – 3.90 (m, 4H), 3.27 (s, 3H), 2.19 (s, 3H), 2.09 (s, 6H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.2, 170.0, 169.7, 165.9, 153.9, 136.3, 131.4, 131.0, 130.5, 129.6, 129.5, 128.5, 127.6, 124.1, 118.0, 100.2, 71.3, 70.6, 67.9, 66.9, 61.5, 43.5, 37.9, 20.8, 20.7, 20.5; HRMS (ESI) for $C_{29}H_{33}NO_{11}S_2$ $[M+Na]^+$ Calculated: 658.1393. Found: 658.1389.

General procedure for synthesis of compounds 1-2: Compounds **9**/**14** were dissolved in anhydrous methanol (3 mL). Sodium methoxide (1 eq) was added to the solution at 0 °C and stirred at room temperature. Upon completion (TLC analysis), the reaction was quenched by addition of amberlite resin. The reaction mixture was filtered through a cotton plug and the filtrate was evaporated under reduced pressure. The crude obtained was purified by reverse phase HPLC using ACN-H_2O as the eluent.

Methyl *N*-acetyl-*S*-((methyl(2-(((2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)carbamoyl)thio)cysteinate (1a):

Compound **9a** (50 mg, 72 μmol) was reacted with sodium methoxide (4 mg, 72 μmol) in methanol at 0 °C and the reaction mixture was stirred for 40 min. The crude was purified to obtain **1a** (17 mg, 50%). FT-IR (ν_{max}; cm^{-1}): 3347, 1666; ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.78 (m, 1H), 7.45 – 7.33 (m, 2H), 7.13 – 7.09 (m, 1H), 5.03 (dd, *J* = 29.2, 7.2 Hz, 1H), 4.75 – 4.63 (m, 2H), 4.00 – 3.79 (m, 1H), 3.68 (d, *J* = 12.4 Hz, 3H), 3.54 – 3.44 (m, 4H), 3.29 (d, *J* = 10.0 Hz, 3H), 3.21 – 3.02 (m, 2H), 1.94 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 166.8, 155.8, 132.0, 131.7, 130.8, 123.6, 118.3, 117.4, 102.2, 78.0, 74.5, 71.1, 62.5, 52.7, 52.0, 41.6, 38.4, 22.6; HRMS (ESI) for $C_{20}H_{28}N_2O_{10}S_2$ $[M+Na]^+$ Calculated: 543.1082, Found: 543.1077.

***SS*-benzyl methyl(2-(((2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)carbamo(dithioperoxoate) (1b):**

Compound **9b** (60 mg, 94 μmol) was reacted with sodium methoxide (5 mg, 94 μmol) in methanol at 0 °C and the reaction mixture was stirred for 40 min. The crude was purified to obtain **1b** (19 mg, 45%). FT-IR (ν_{max}; cm^{-1}): 3370, 1660; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.25 (m, 8H), 7.08 (q, *J* = 6.8 Hz, 1H), 4.95 (dd, *J* = 28.0, 7.2 Hz, 1H), 3.96 – 3.87 (m, 3H), 3.73 – 3.64 (m, 1H), 3.49 – 3.37 (m, 5H), 3.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 156.1, 137.8, 132.4, 131.8, 130.6, 129.5, 128.6, 123.8, 117.8, 102.6, 78.5, 78.2, 74.9, 71.3, 62.6, 44.2, 38.6; HRMS (ESI) for $C_{21}H_{25}NO_7S_2$ $[M+H]^+$ Calculated: 468.1150, Found: 468.1159.

***SS*-benzyl methyl(2-(((2*S*,3*S*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)carbamo(dithioperoxoate) (2b):**

Compound **14b** (100 mg, 157 μmol) was reacted with sodium methoxide (8.5 mg, 157 μmol) in methanol at 0 °C and the reaction mixture was stirred for 1 h. The crude was purified to obtain **2b** (23 mg, 31%). FT-IR (ν_{max}; cm^{-1}): 3367, 1665; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.24 (m, 7H), 7.09 –7.05 (m, 1H), 4.95 (dd, *J* = 25.0, 8.0 Hz, 1H), 3.98 – 3.81 (m, 10H), 3.65 – 3.62 (m, 1H), 3.31 (d, *J* = 9.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 156.0, 137.7, 131.8, 130.4, 129.3, 128.2, 123.5, 117.8, 102.7, 78.2, 78.0, 74.5, 71.3, 62.6, 43.9, 38.3; HRMS (ESI) for $C_{21}H_{25}NO_7S_2$ $[M+H]^+$ Calculated: 468.1150, Found: 468.1141.

Experimental Procedures

Persulfide/polysulfide measurement from 1a and 1b using LC/MS: Stock solutions of **1a** (20 mM), **1b** (20 mM) and HPE-IAM (125 mM) were prepared in DMSO. Stock solution of ®-glucosidase (90 U/mL) was prepared in DI water. The reaction mixture for **1a** and **1b** was prepared by adding 100 μM of the compounds **1a** or **1b** (5 μL, 20 mM stock) along with 2 mM HPE-IAM (16 μL, 125 mM) and 10 U/mL ®-glucosidase (111 μL, 90 U/mL stock). The volume was adjusted to 1 mL using 20 mM phosphate buffer, pH 7.4 and the reaction mixture was incubated at 37 °C. 100 μL aliquots of the reaction mixture was taken at pre-determined time points and the reaction was quenched by adding 100 μL of methanol. The samples were centrifuged at 10000g for 10 min at 4 °C, the supernatant was collected and assessed thereafter by LC/MS. All measurements were done using a previously established LC/MS method[4] with slight modification. Measurements were carried out in the positive ion mode using high resolution multiple reaction monitoring (MRM-HR) analysis on a Sciex X500R quadrupole time-of flight (QTOF) mass spectrometer fitted with an Exion UHPLC system using a Kinetex 2.6 mm hydrophilic interaction liquid chromatography

(HILIC) column with 100 Å particle size, 150 mm length and 3 mm internal diameter (Phenomenex). Acetonitrile (A) and 0.1% formic acid in water (B) was used as the mobile phase. Nitrogen was the nebulizer gas, with the nebulizer pressure set at 50 psi. The MRM-HR mass spectrometry parameters for measuring polysulfides are: m/z precursor ion mass (Q1, $M + H^+$)/ product ion mass (Q3, $M + H^+$) 521.13/105.05 (**1a**), 150.06/76.00 (**3**), 387.11/121.10 (**15a**), 419.08/121.10 (**15b**), 451.05/121.10 (**15c**), 389.16/121.10 (**16a**), 421.13/121.10 (**16b**), 453.09/121.1 (**16c**), 468.12/105.05 (**1b**), 334.10/121.10 (BnSS-HPE-AM), 366.06/121.10 (BnSSS-HPE-AM), 398.04/121.10 (BnSSSS-HPE-AM), declustering potential =80 V, entrance potential = 10 V, collision energy = 20 V, and collision exit potential = 5 V.

Persulfide/polysulfide measurement from 2b using LC/MS: Stock solutions of **2b** (20 mM) and HPE-IAM (125 mM) were prepared in DMSO. Stock solution of ®-galactosidase (100 U/mL) was prepared in DI water. The reaction mixture for **2b** was prepared by adding 100 μM of **2b** (2.5 μL, 20 mM stock), 2 mM HPE-IAM (8 μL, 125 mM), 2 U/mL ®-galactosidase (10 μL, 100 U/mL stock) and the volume was adjusted to 500 μL using 20 mM phosphate buffer, pH 7.4. The reaction mixture was incubated at 37 °C. 100 μL aliquots of the reaction mixture was taken at pre-determined time points and the reaction was quenched by adding 100 μL of methanol. The samples were centrifuged at 10000g for 10 min at 4 °C, the supernatant was collected and assessed thereafter by LC/MS using the method described above.

Decomposition of 2b in the presence of NAC: Stock solution of **2b** (20 mM) was prepared in DMSO. ®-galactosidase (100 U/mL) and *N*-acetyl cysteine (NAC, 100 mM) were prepared in DI water. The reaction mixture was prepared by adding 100 μM of **2b** (2.5 μL, 20 mM stock), 2 U/mL ®-galactosidase (10 μL, 100 U/mL stock) and 0.5 mM NAC (2.5 μL, 100 mM stock). The volume was adjusted to 0.5 mL using 20 mM phosphate buffer, pH 7.4 and the reaction was incubated at 37 °C. After 30 min, 100 μL aliquots of the reaction mixture was taken and was quenched by adding 100 μL of methanol. The samples were centrifuged at 10000g for 10 min at 4 °C and the supernatants were collected and assessed thereafter by LC/MS using the method described above.

Decomposition of 2b in the presence of GSH: Stock solution of **2b** (20 mM) was prepared in DMSO. ®-galactosidase (100 U/mL) and glutathione (GSH, 100 mM) were prepared in DI water. The reaction mixture was prepared by adding 100 μM of **2b** (2.5 μL, 20 mM stock), 2 U/mL ®-galactosidase (10 μL, 100 U/mL stock) and 1 mM GSH (5 μL, 100 mM stock). The volume was

adjusted to 0.5 mL using 20 mM phosphate buffer, pH 7.4 and the reaction was incubated at 37 °C. After 30 min, 100 μL aliquots of the reaction mixture was taken and was quenched by adding 100 μL of methanol. The samples were centrifuged at 10000g for 10 min at 4 °C and the supernatants were collected and assessed thereafter by LC/MS using the method described above.

Cell viability assay: Human colon adenocarcinoma cells (DLD-1) and hepatocarcinoma cells (HepG2) were seeded at a concentration of 1×10^4 cells/well in a 96-well plate in Roswell Park Memorial Institute (RPMI) media supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic solution and incubated in an atmosphere of 5% CO_2 at 37 °C for 16 h. Following this, the cells were exposed to varying concentrations of the compounds. Stock solutions of compounds were prepared in DMSO and the final concentration of DMSO did not exceed 0.5%. The cells were then incubated for 24 h at 37 °C. A 0.5 mg/mL stock solution of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was prepared in RPMI. The old media from the cells were removed, 100 μL of the MTT stock was added to each well and incubated for 4 h at 37 °C. After 4 h incubation, the media was carefully removed and 100 μL of DMSO was added. Spectrophotometric analysis of each well at 570 nm using a microplate reader (Thermo Scientific Varioskan) was carried out to estimate cell viability.

Detection of persulfides/polysulfides in cells using SSP2:[5,6] DLD-1 cells were seeded in a 12 well plate with 10^5 cells/well in RPMI media supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic solution and incubated in an atmosphere of 5% CO_2 at 37 °C for 48 h. After incubation, the old media was removed and the cells were washed with 500 μL PBS. This was followed by addition of 1 mL fresh serum free RPMI media containing SSP2 (50 μM) along with cetyltrimethylammonium bromide (CTAB, 500 μM) and the cells were incubated at 37 °C for 20 mins. The media was removed and cells were washed with serum free media to remove the excess probe. This was followed by incubation with **1a** (100 μM, 500 μM) in serum free media for 1 h at 37 °C. Cells were finally washed twice with PBS and were imaged on EVOS fluorescence microscope using GFP (green fluorescence protein) filter.

Protection from oxidative stress:

DLD-1 cells: DLD-1 were seeded in a 96-well plate with 10^4 cells/well in RPMI media supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic solution and incubated in an atmosphere of 5% CO_2 at 37 °C for 16 h. Stock solutions of compounds were prepared in DMSO with final concentration of DMSO not exceeding 0.5%. After 16 h, the cells were pre-treated with different concentrations of the compound for 12 h followed by treatment with MGR-1 (35 µM) for 4 h at 37 °C. The media was removed and MTT assay was carried out as described above to determine cell viability.

HepG2 cells: HepG2 were seeded in a 96-well plate with $2.5 \cdot 10^4$ cells/well in RPMI media supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic solution and incubated in an atmosphere of 5% CO_2 at 37 °C for 16 h. Stock solutions of compounds were prepared in DMSO with final concentration of DMSO not exceeding 0.5%. After 16 h, the cells were pre-treated with different concentrations of the compound for 12 h followed by treatment with MGR-1 (20 µM) for 4 h at 37 °C. The media was removed and MTT assay was performed as described above to determine cell viability.

Supplementary Figures:

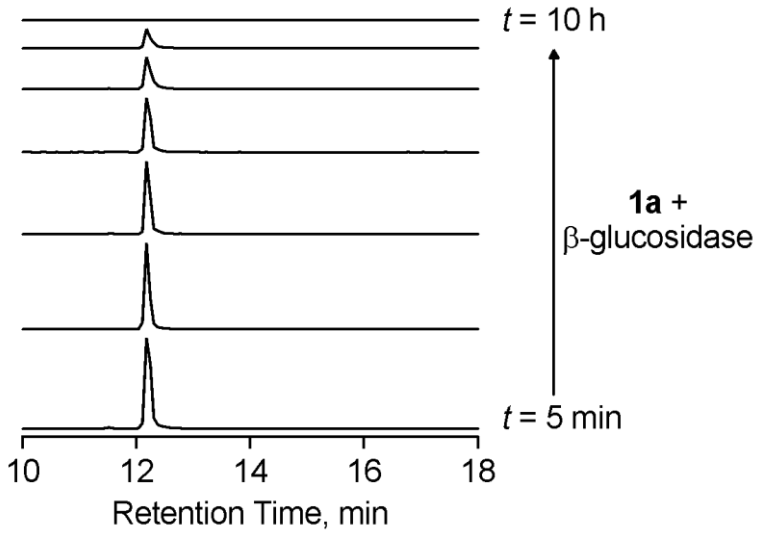


Figure S1: Extracted ion chromatograms for **1a** (m/z = 521.125; expected m/z = 521.1264) at different time points (5, 30, 60, 120, 210, 300, 600 min). Decomposition of **1a** over 10 h in the presence of ®-glucosidase was observed.

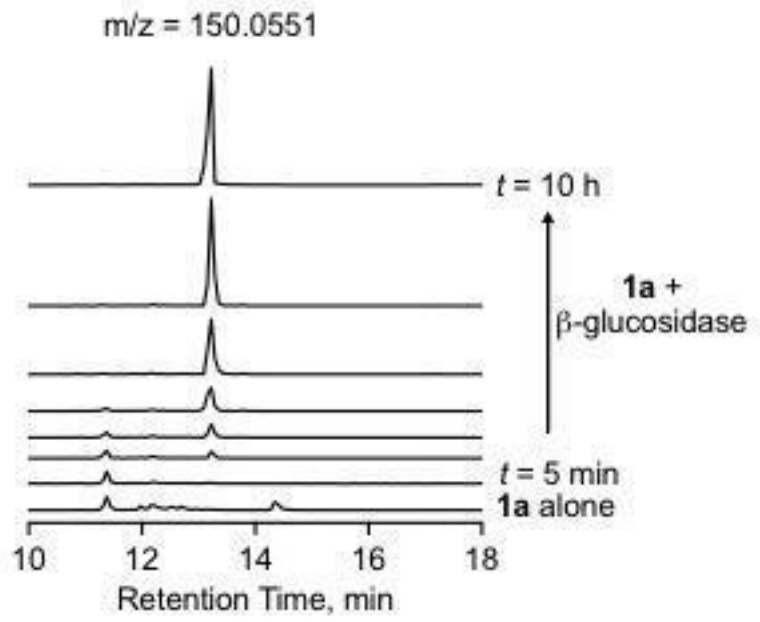


Figure S2: Extracted ion chromatograms for the formation of by-product (**3**) (m/z = 150.0551; expected m/z = 150.0555) at given time points (5, 30, 60, 120, 210, 300, 600 min). Formation of **3** over 10 h in the presence of ®-glucosidase was observed.

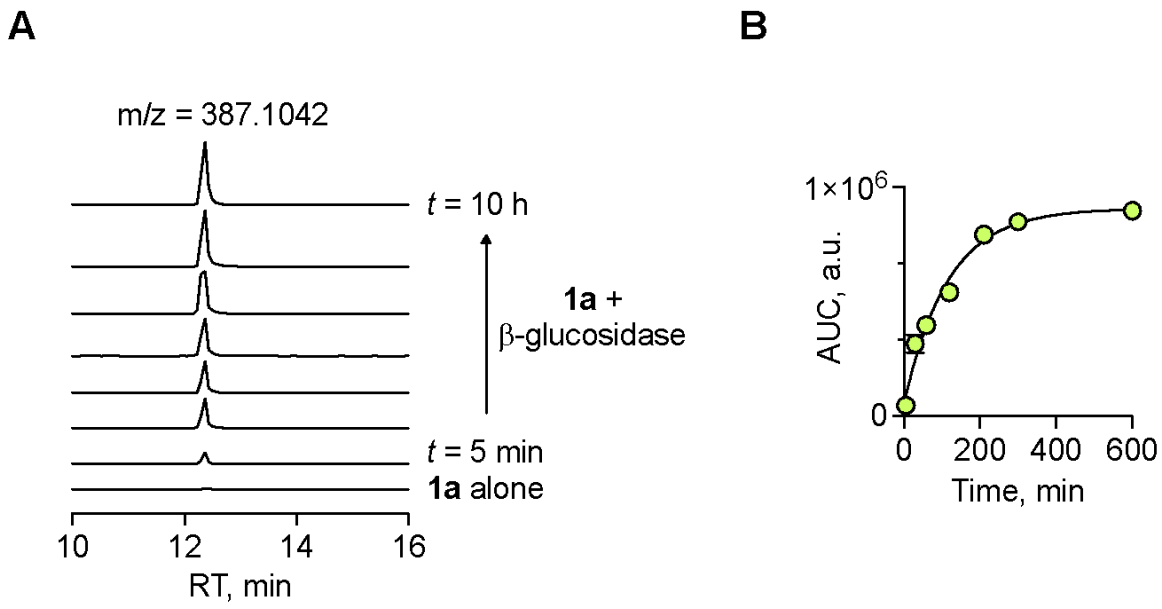


Figure S3. a) Extracted ion chromatograms for the formation of the persulfide adduct of HPE-IAM (**15a**) (m/z = 387.1042; expected m/z = 387.1048) at the given time points (5, 30, 60, 120, 210, 300, 600 min). Formation of **15a** over 10 h in the presence of ®-glucosidase was observed. b) Curve fitting to first order gave a rate constant of 0.0054 min^{-1} (R^2 = 0.9978).

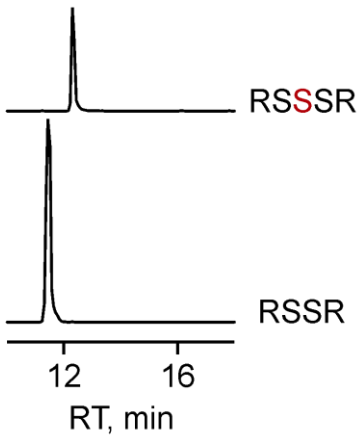


Figure S4: Disulfide and trisulfide (R = *N*-acetylcysteine methylester, NACMe) formed upon incubation of **1a** in the presence of ®-glucosidase. A peak for disulfide was obtained at RT 11.4 min with an observed m/z = 353.0849 $[M + H]^+$ (expected m/z = 353.0841). A peak for the trisulfide was obtained at RT 12.3 min with an observed m/z = 385.0560 $[M + H]^+$ (expected m/z = 385.0562).

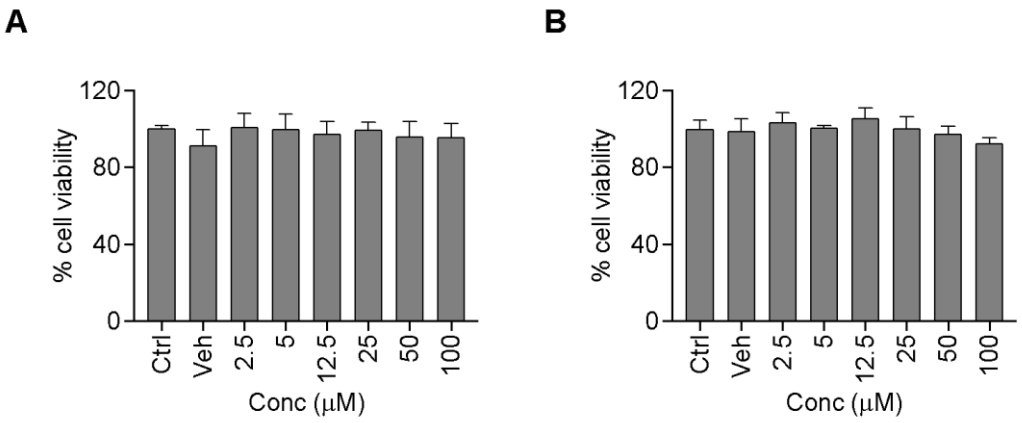


Figure S5: a) Cell viability assay carried out with the compound **1a** for 24 h on a) DLD-1 cells b) HepG2 cells. No significant change in viable cells upon treatment with the compound compared with the control was observed. All data are presented as mean ± SD (n =3/group).

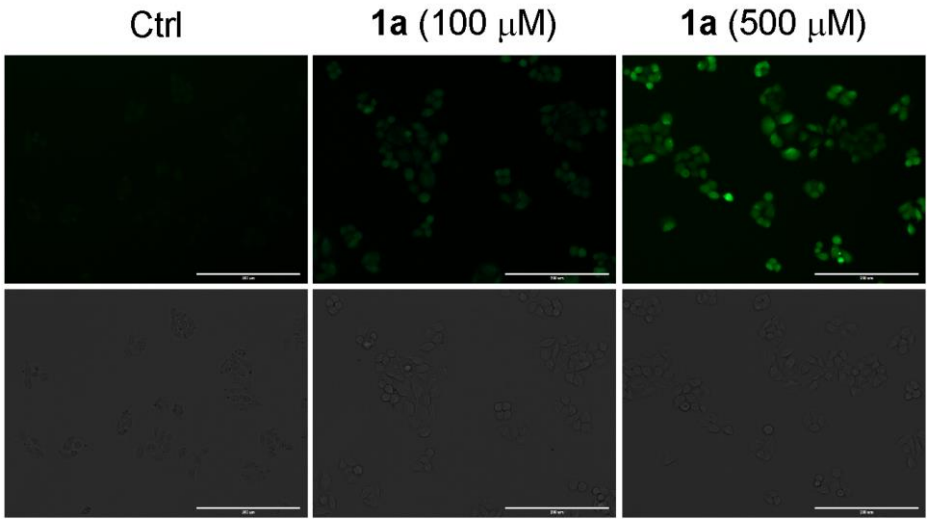


Figure S6: Detection of persulfides/polysulfides in colon carcinoma cells (DLD-1) using the probe SSP-2. Cells were pre-incubated with SSP2 (50 μM) and CTAB (500 μM) for 20 mins followed by treatment of **1a** (100 μM and 500 μM) for 1 h. Ctrl refers to untreated cells. Scale bar represents 200 mm. The cells were imaged in the 20· GFP filter.

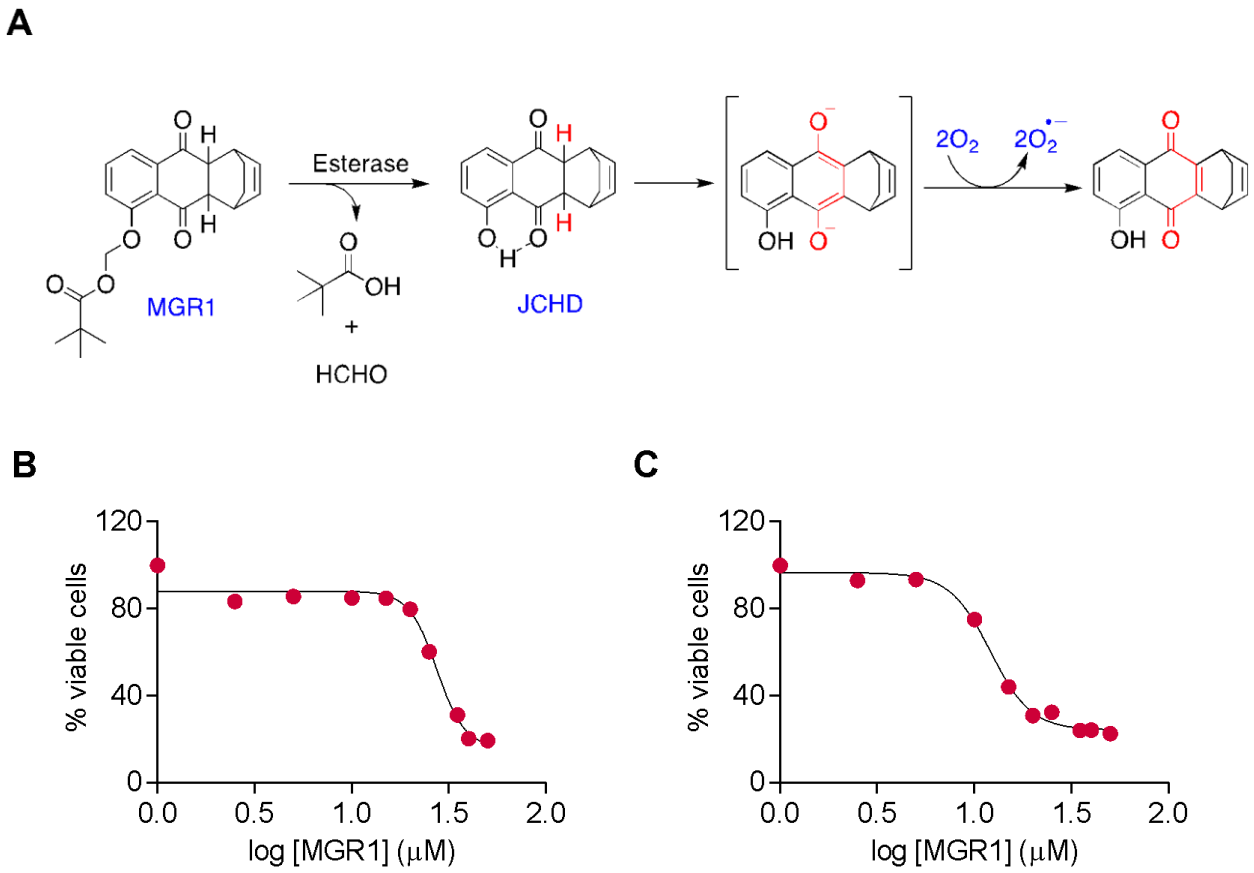


Figure S7: a) Mechanism for esterase activated ROS generation from MGR1. MGR1 after entry into mammalian cells gets acted upon by esterase, producing the active ROS generator JCHD.[7,8] b) Cell viability assay carried out with the MGR-1 for 4 h on DLD-1 cells to determine its IC_{50}. IC_{50} was calculated to be 27 μM. All data are presented as mean ± SD (n =3/group). c) Cell viability assay carried out with the MGR-1 for 4 h on HepG2 cells. IC_{50} was calculated to be 12 μM.

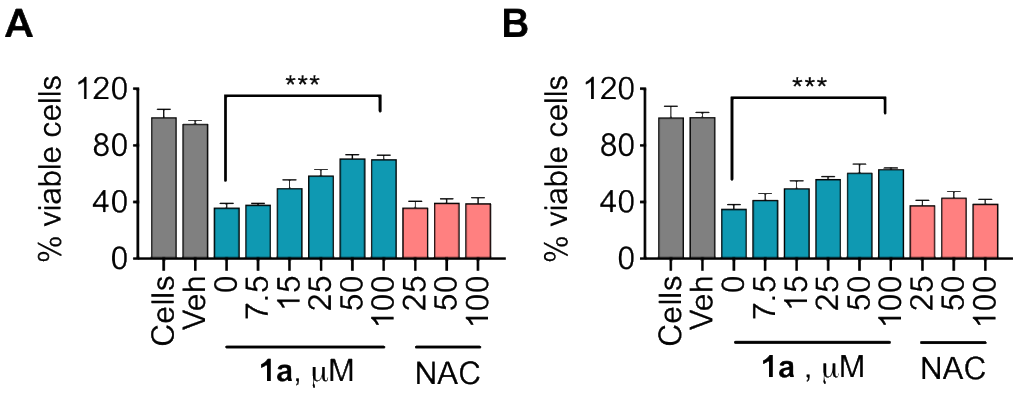


Figure S8: a) Cytoprotective effects of **1a** against ROS induced cell death. Colon carcinoma (DLD-1) cells were pre-treated with varying doses of **1a** for 12 h followed by treatment with the cell permeable ROS generator MGR-1 (20 □M) for 4h. A similar experiment was carried out with N-acetylcysteine (NAC). No significant effects on cell viability was observed with NAC. Results are presented as mean ± SD (n = 3). (***) $p < 0.001$ *vs* MGR-1. c) A similar experiment carried out on the liver cell line (HepG2). A dose dependant increment in cell viability was observed upon pre-treatment with **1a**, against cell death induced by MGR-1. No significant effects on cell viability was obtained with NAC. Results are presented as mean ± SD (n = 3). (***) $p < 0.001$ *vs* MGR-1.

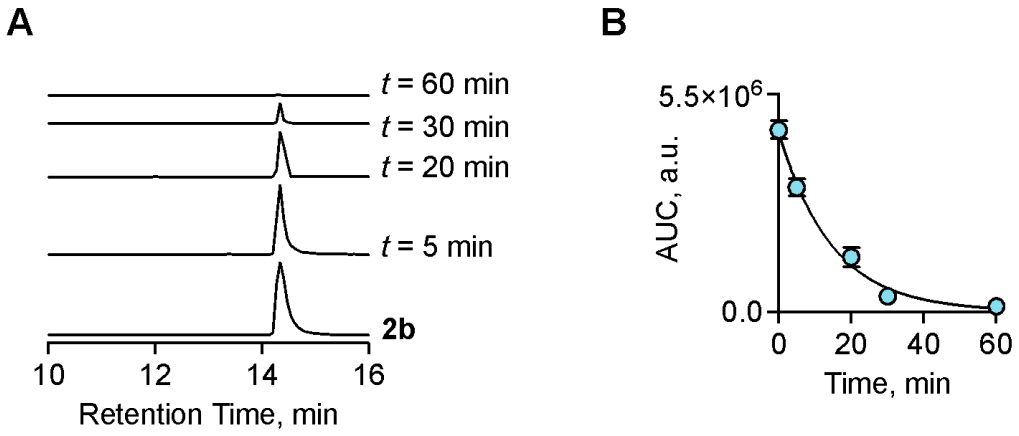


Figure S9: a) Extracted ion chromatograms for **2b** (m/z = 468.1146; expected m/z = 468.1151) at different time points. Decomposition of **2b** over 60 min in the presence of ®-galactosidase was observed. b) Curve fitting to first order gave a rate constant of 0.0684 min^{-1} (R^2 = 0.9929).

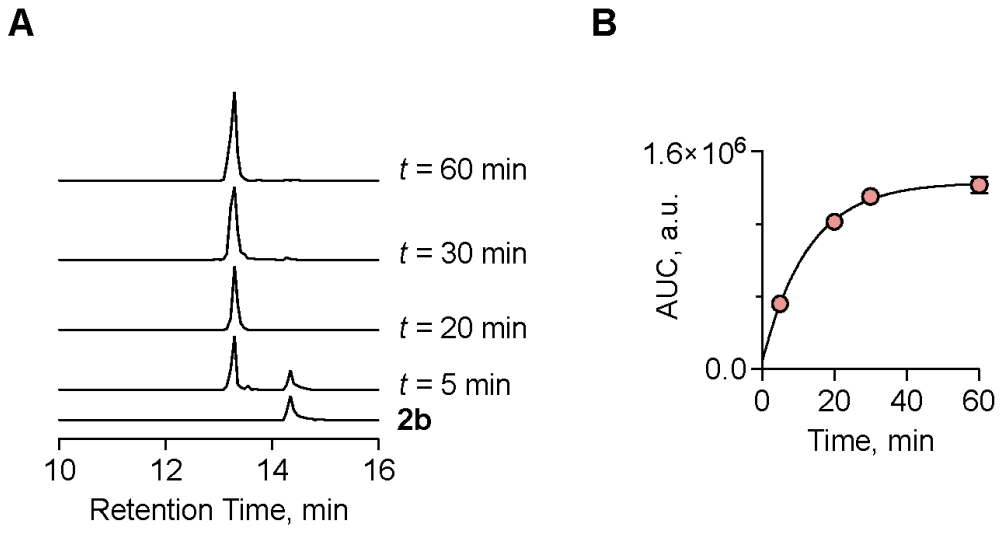


Figure S10: a) Extracted ion chromatograms for the formation of by-product (**3**) (m/z = 150.0545; expected m/z = 150.0555) at given time points. Formation of **3** over 60 min in the presence of ®-galactosidase was observed. b) Curve fitting to first order gave a rate constant of 0.0858 min^{-1} (R^2 = 0.999).

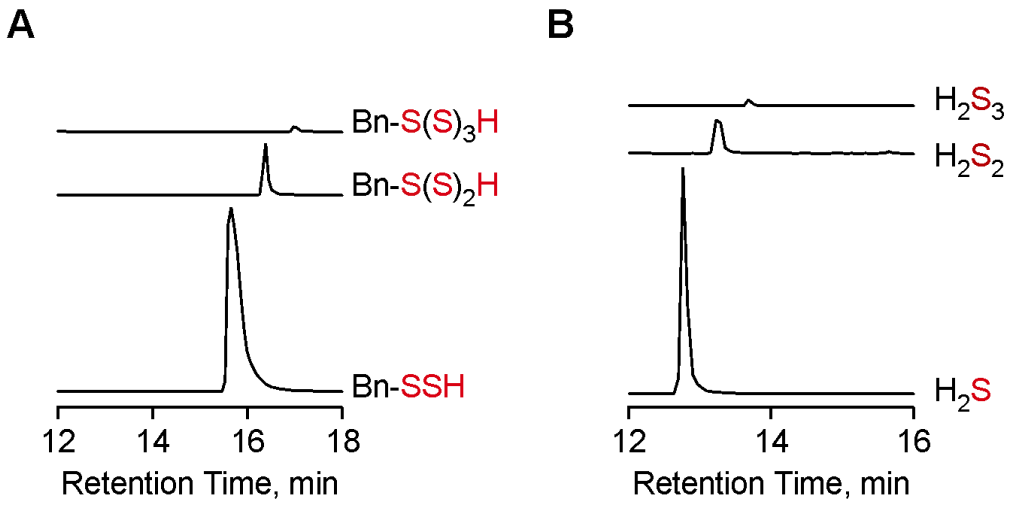


Figure S11: a) Extracted ion chromatogram of benzyl persulfides and polysulfides generated from compound **2b**, detected as their HPE-AM adducts using LC/MS. Bn-SS-HPE-AM (m/z = 334.0915 $[M+H]^+$; expected, 334.0935); Bn-$S(S)_2$-HPE-AM (m/z = 366.0659 $[M+H]^+$; expected, 366.0656); Bn-$S(S)_3$-HPE-AM (m/z = 398.0379 $[M+H]^+$; expected, 398.0377). b) Extracted ion chromatogram of hydrogen sulfide and hydrogen polysulfides detected as their bis-HPE-AM adducts. Bis-S-HPE-AM (m/z = 389.1540 $[M+H]^+$; expected, 389.1535); bis-S_2-HPE-AM (m/z = 421.1258 $[M+H]^+$; expected, 421.1256); bis-S_3-HPE-AM (m/z = 453.0980 $[M+H]^+$; expected, 453.0976).

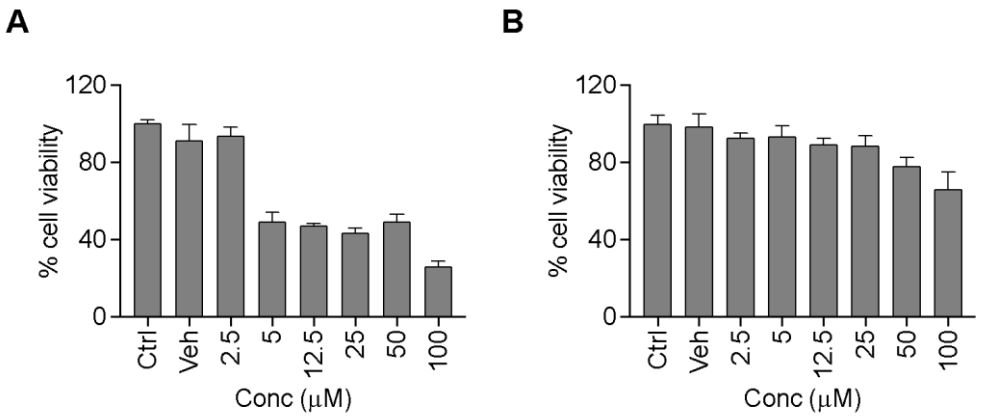


Figure S12: Cell viability assay carried out with the compound **2b** for 24 h on a) DLD-1 cells b) HepG2 cells. All data are presented as mean ± SD (n =3/group).

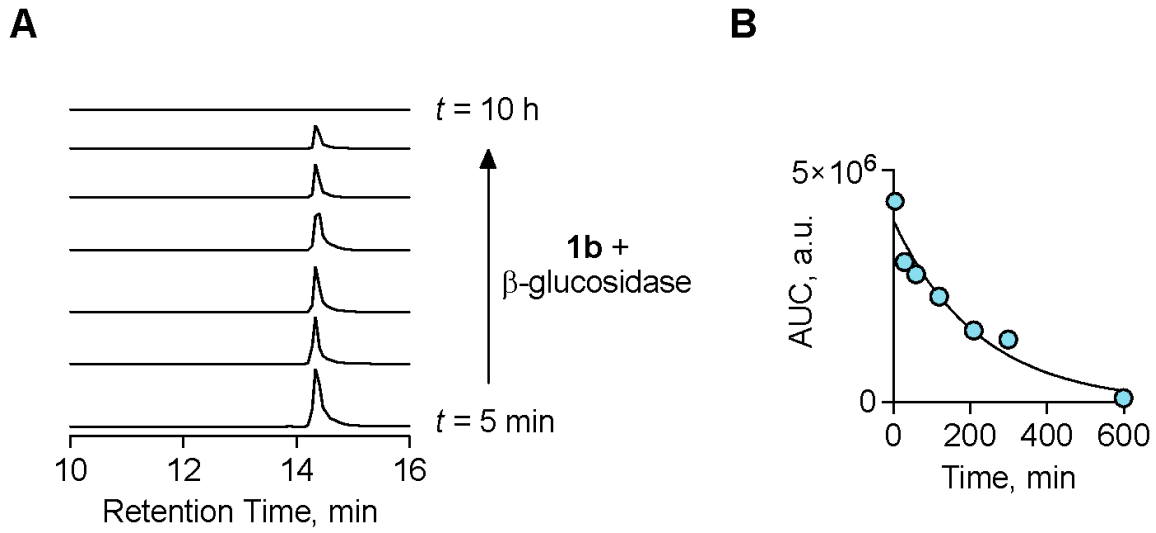


Figure S13: a) Extracted ion chromatograms for **1b** (m/z = 468.1153; expected m/z = 468.1151) at different time points (5, 30, 60, 120, 210, 300, 600 min). Decomposition of **1b** over 10 h in the presence of ®-glucosidase was observed. b) Curve fitting to first order gave a rate constant of 0.00436 min^{-1} (R^2 = 0.958).

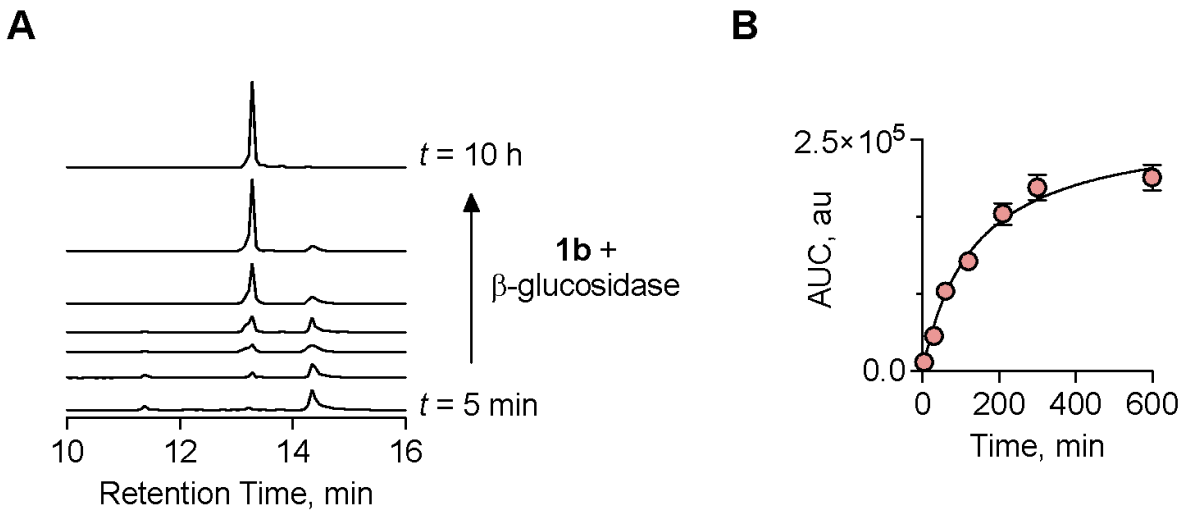


Figure S14: a) Extracted ion chromatograms for the formation of by-product (**3**) (m/z = 150.0545; expected m/z = 150.0555) at given time points (5, 30, 60, 120, 210, 300, 600 min). Formation of **3** over 10 h in the presence of ®-glucosidase was observed. b) Curve fitting to first order gave a rate constant of 0.0074 min^{-1} (R^2 = 0.9936).

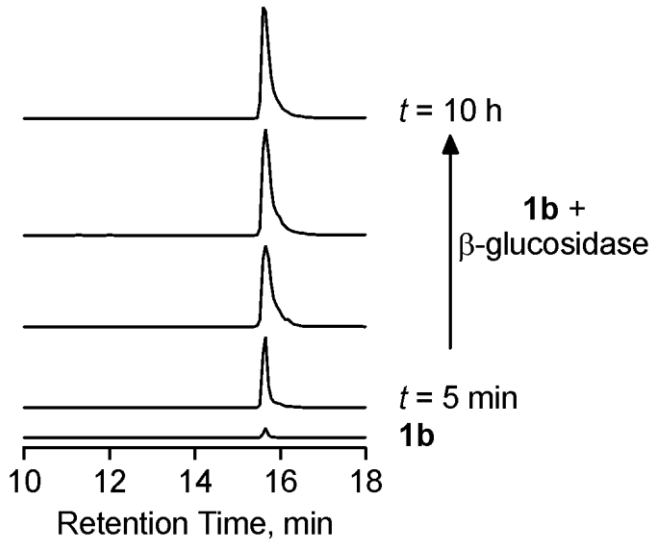


Figure S15: Extracted ion chromatograms for the formation of the benzyl persulfide adduct of HPE-IAM (BnSS-HPE-AM) (m/z = 334.0922; expected m/z = 334.0935) at the given time points (5, 30, 60, 120, 210, 300, 600 min). Formation of BnSSH over 10 h in the presence of ®-glucosidase was observed.

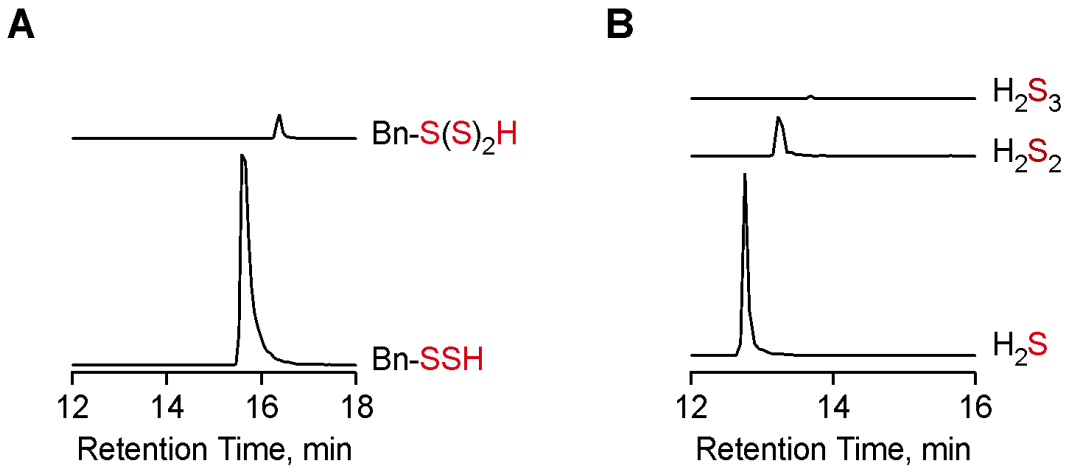


Figure S16: a) Extracted ion chromatogram of benzyl persulfides and polysulfides generated from compound **1b**, detected as their HPE-AM adducts using LC/MS. Bn-SS-HPE-AM (m/z = 334.0922 $[M+H]^+$; expected, 334.0935); Bn-S(S)$_2$-HPE-AM (m/z = 366.0654 $[M+H]^+$; expected, 366.0656). b) Extracted ion chromatogram of hydrogen sulfide and hydrogen polysulfides detected as their bis-HPE-AM adducts. Bis-S-HPE-AM (m/z = 389.1539 $[M+H]^+$; expected, 389.1535); bis-S$_2$-HPE-AM (m/z = 421.1264 $[M+H]^+$; expected, 421.1256); bis-S$_3$-HPE-AM (m/z = 453.0988 $[M+H]^+$; expected, 453.0976).

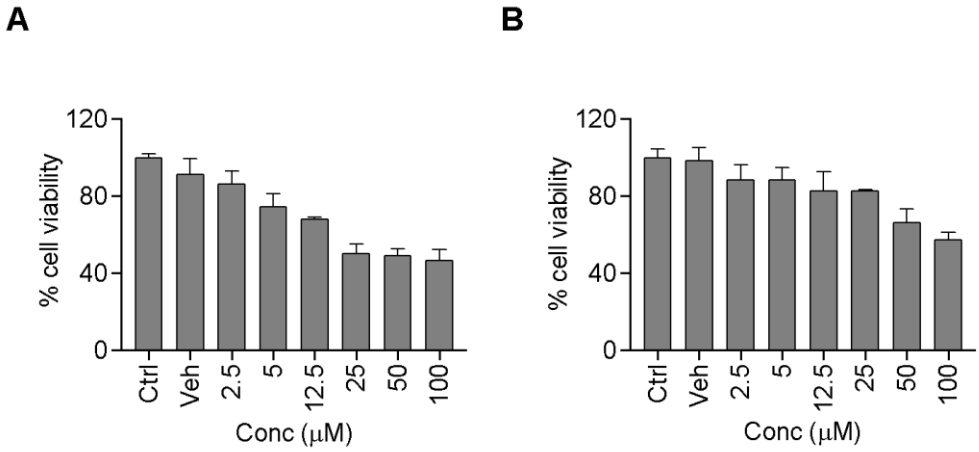


Figure S17: Cell viability assay carried out with the compound **1b** for 24 h on a) DLD-1 cells b) HepG2 cells. All data are presented as mean ± SD (n =3/group).

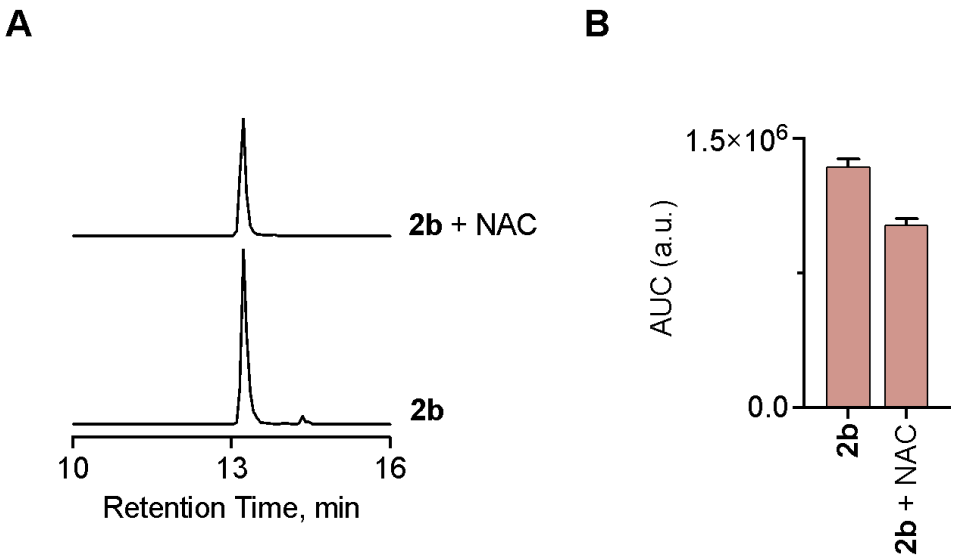


Figure S18: a) Extracted ion chromatograms for the formation of by-product (**3**) (m/z = 150.0545; expected m/z = 150.0555) upon incubation of **2b** with ®-galactosidase in the presence or absence of NAC. b) Quantification of the area under the curve (AUC) for the peak corresponding to **3**. 25% reduction in the yield of **3** was observed upon incubation with NAC.

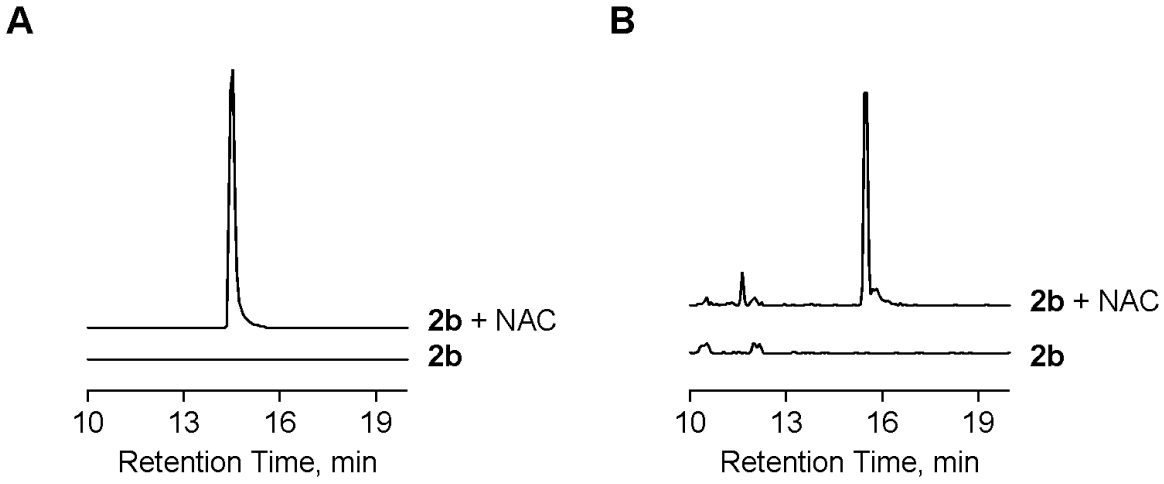


Figure S19: a) Extracted ion chromatograms for the formation of mixed disulfide NAC-SS-Bn (m/z = 150.0545; expected m/z = 286.0572) upon incubation of **2b** with ®-galactosidase in the presence of NAC. b) Extracted ion chromatograms for the formation of mixed trisulfide NAC-SSS-Bn (m/z = 318.0309; expected m/z = 318.0292) upon incubation of **2b** with ®-galactosidase in the presence of NAC.

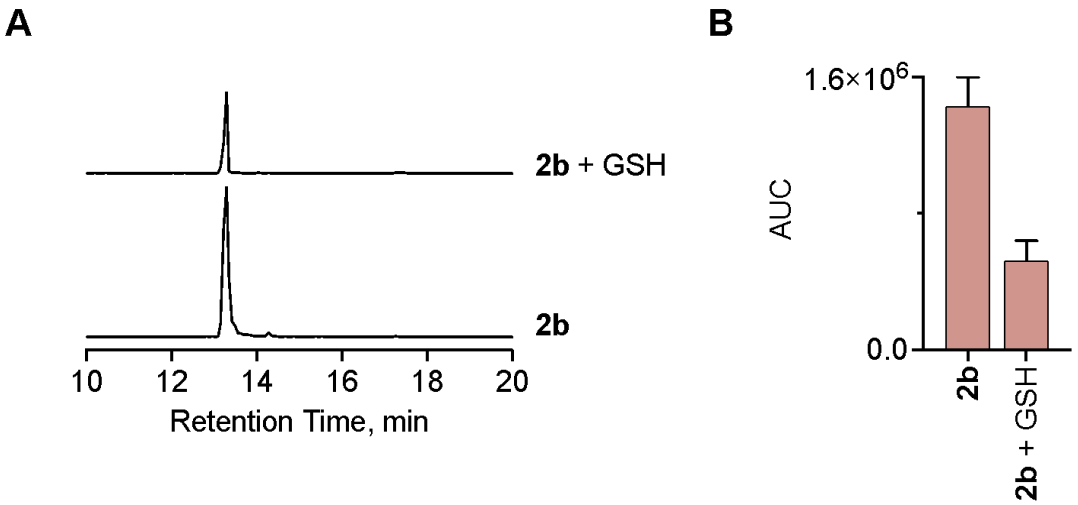


Figure S20: a) Extracted ion chromatograms for the formation of by-product (**3**) (m/z = 150.0558; expected m/z = 150.0555) upon incubation of **2b** with ®-galactosidase in the presence or absence of GSH. b) Quantification of the area under the curve (AUC) for the peak corresponding to **3**. 65% reduction in the yield of **3** was observed upon incubation with GSH.

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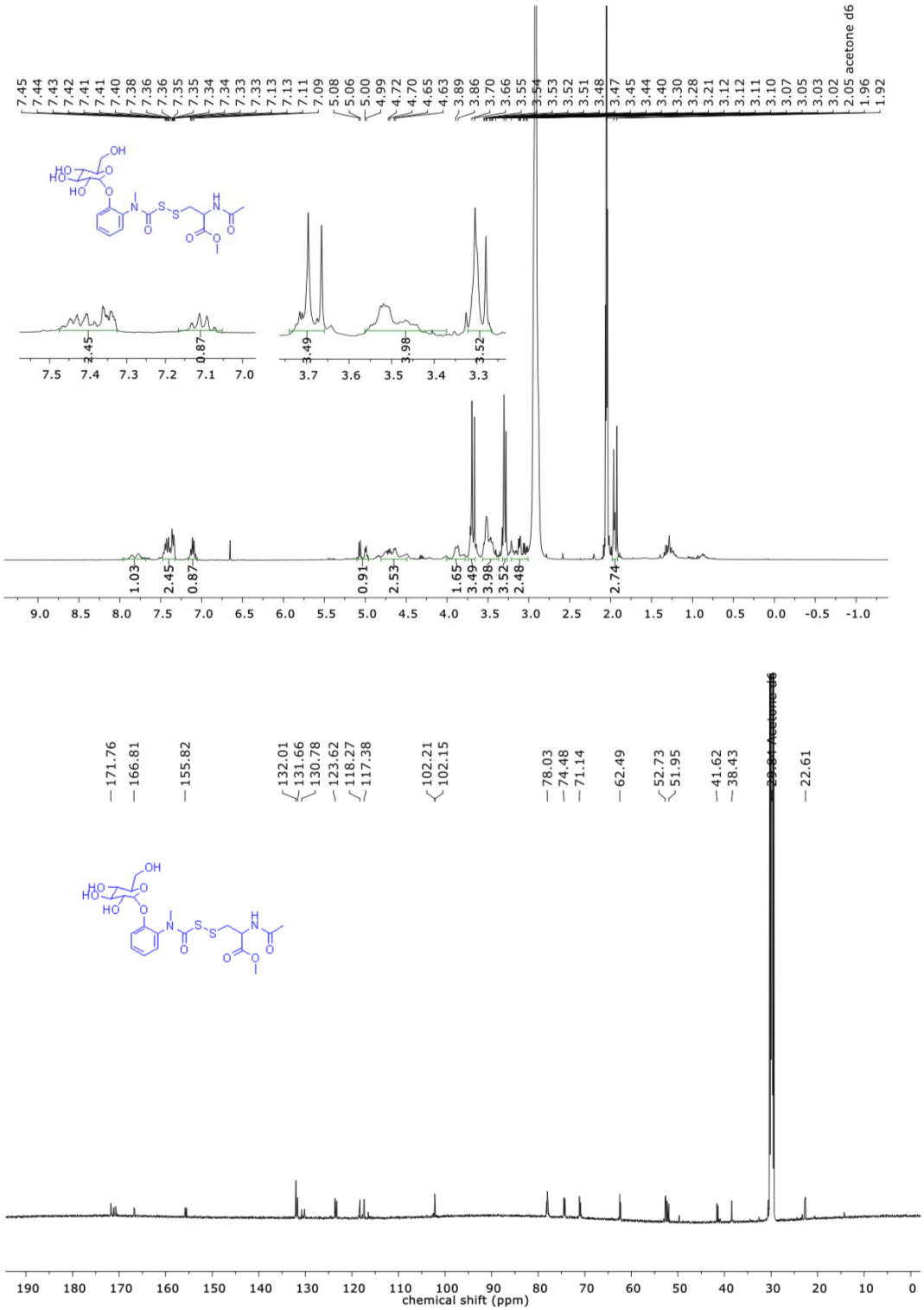
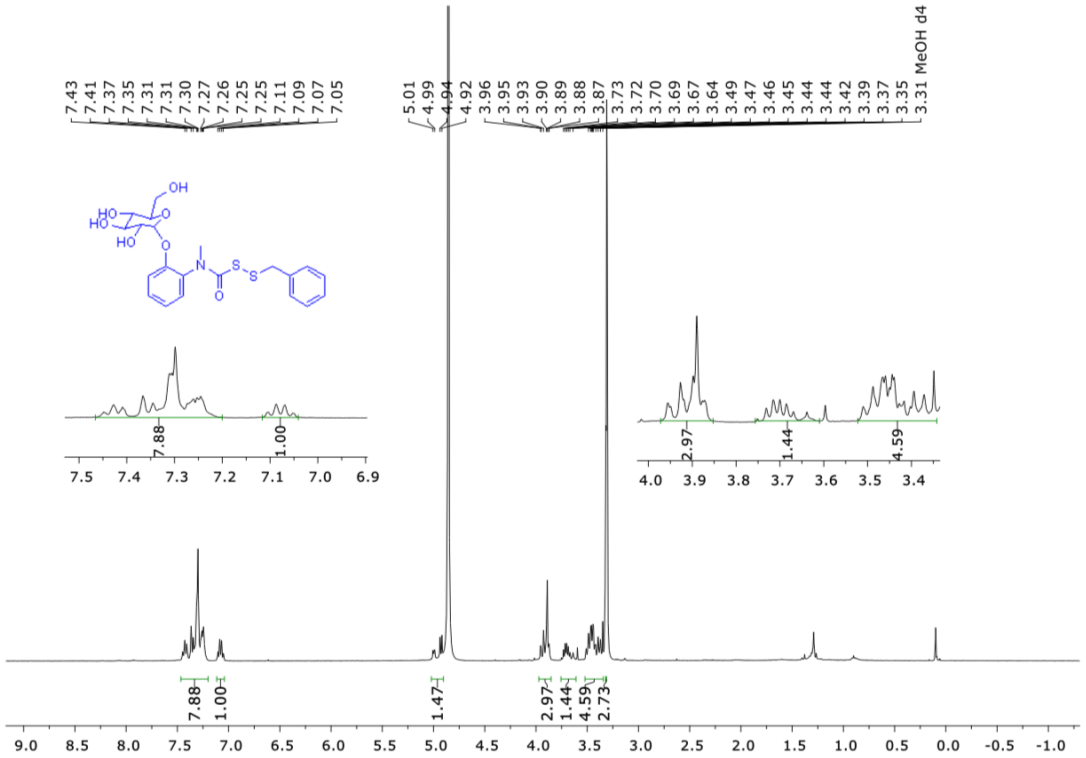
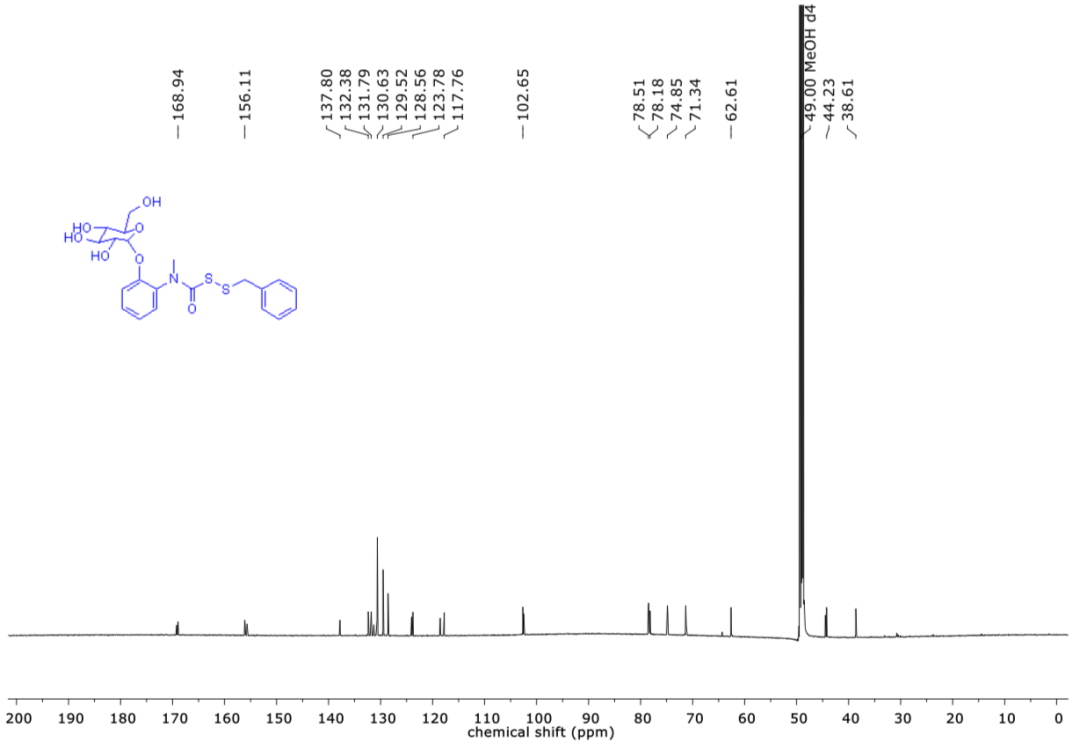
NMR spectra of compounds

^{1}H and ^{13}C NMR spectra of **1a**

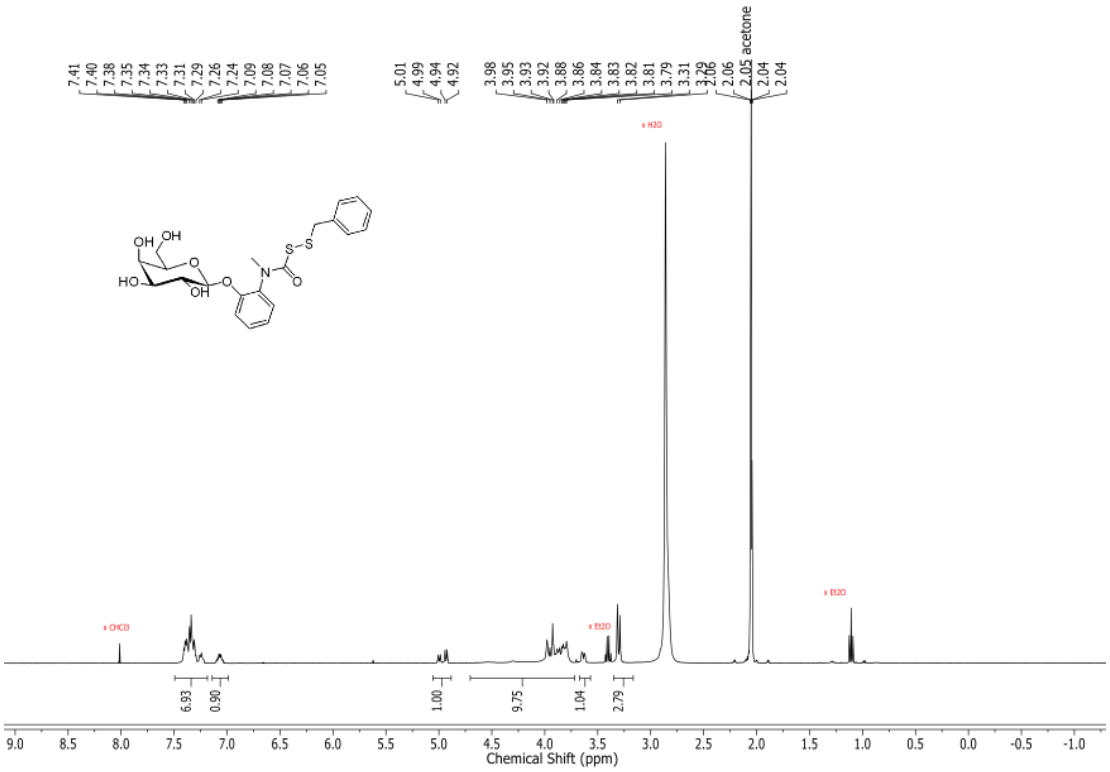


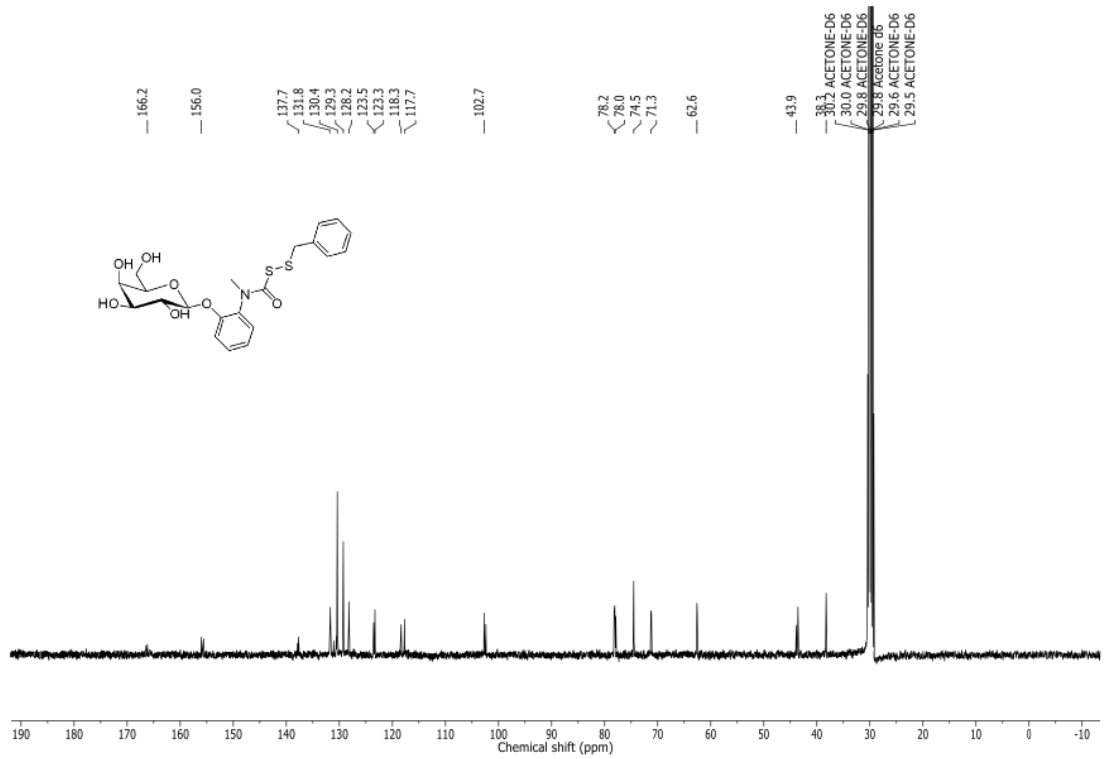
^{1}H and ^{13}C NMR spectra of **1b**



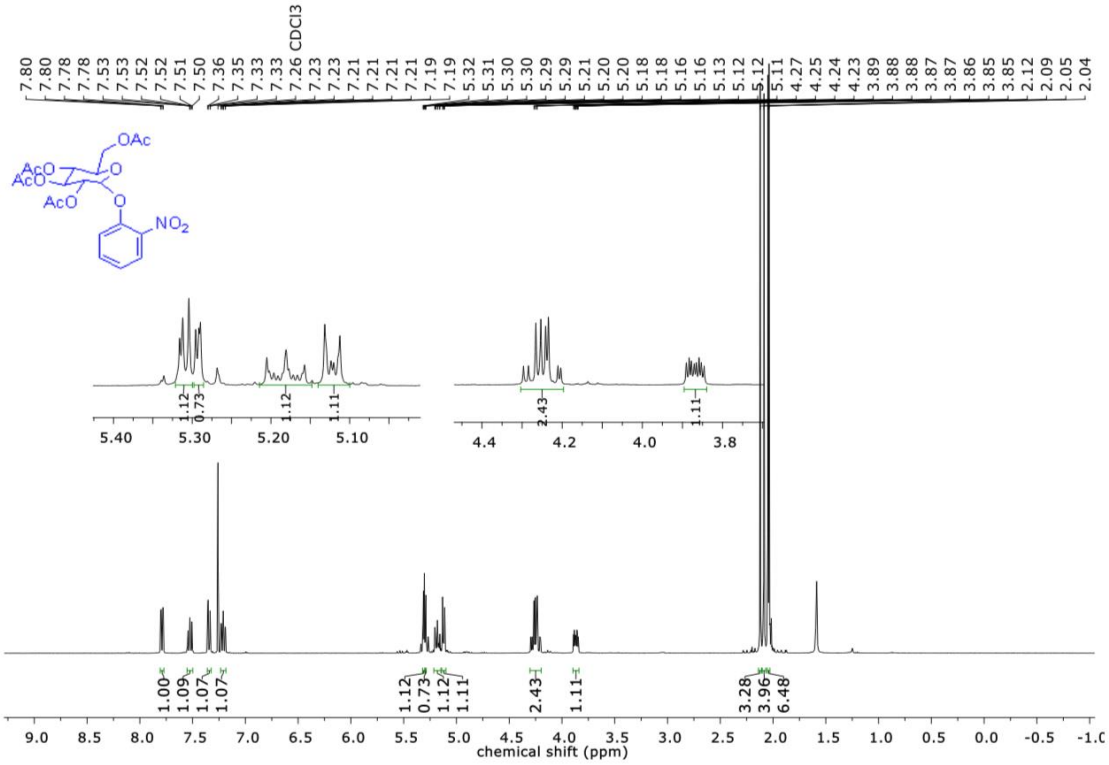


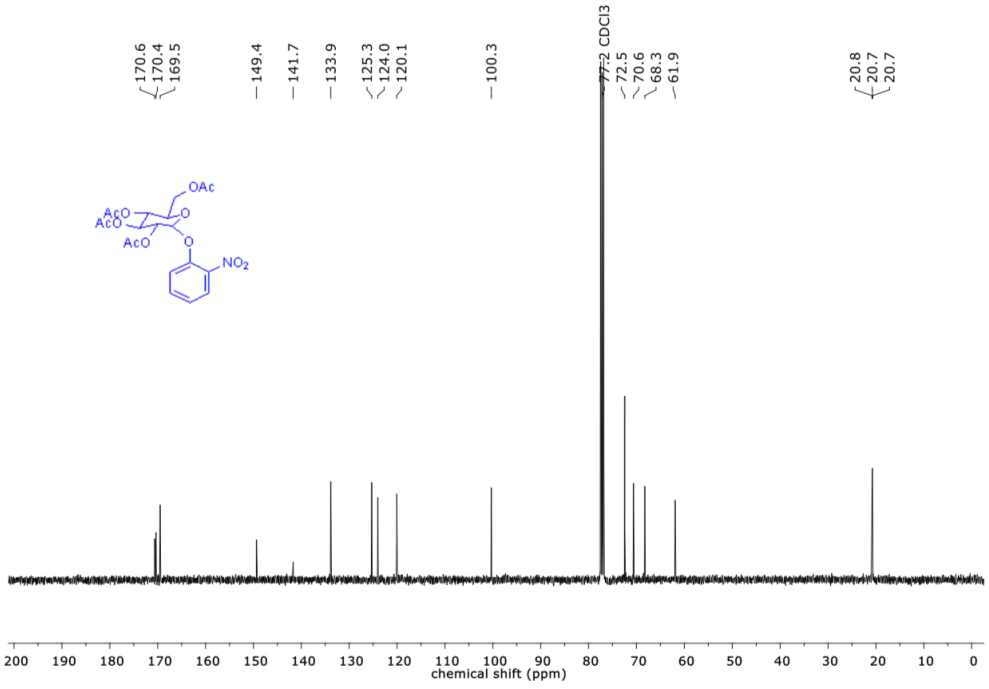
^{1}H and ^{13}C NMR spectra of **2**



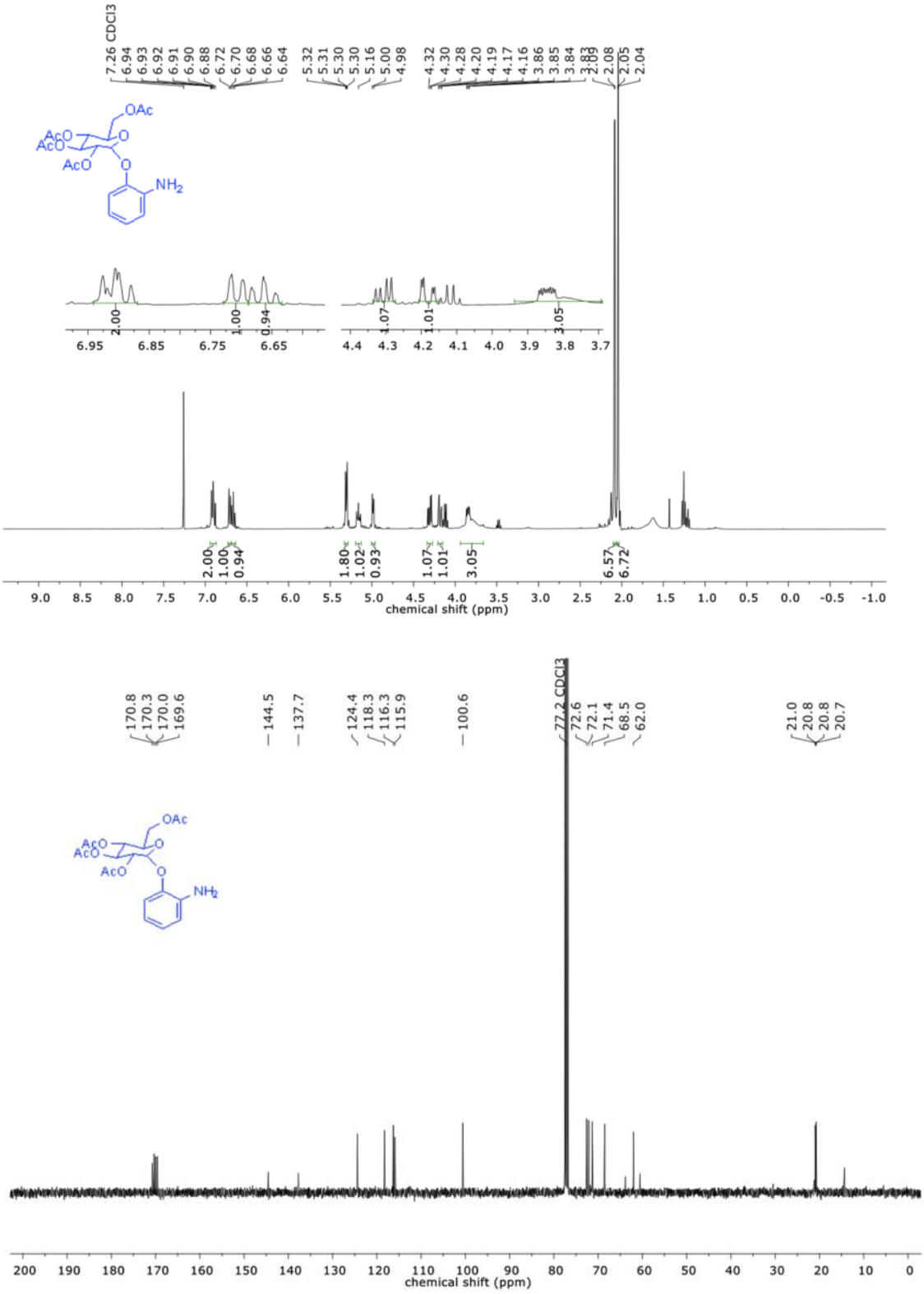


^{1}H and ^{13}C NMR spectra of **5**

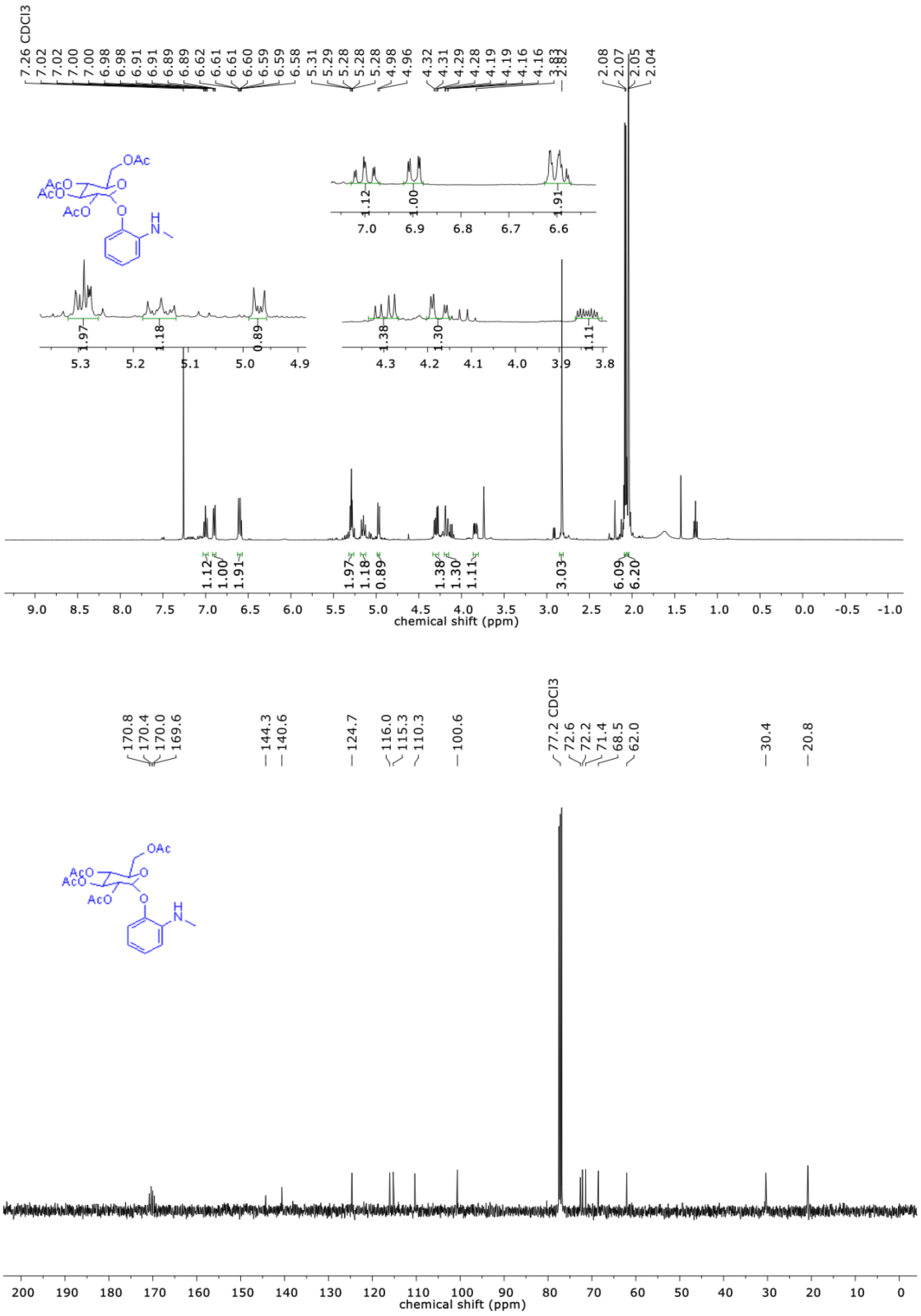




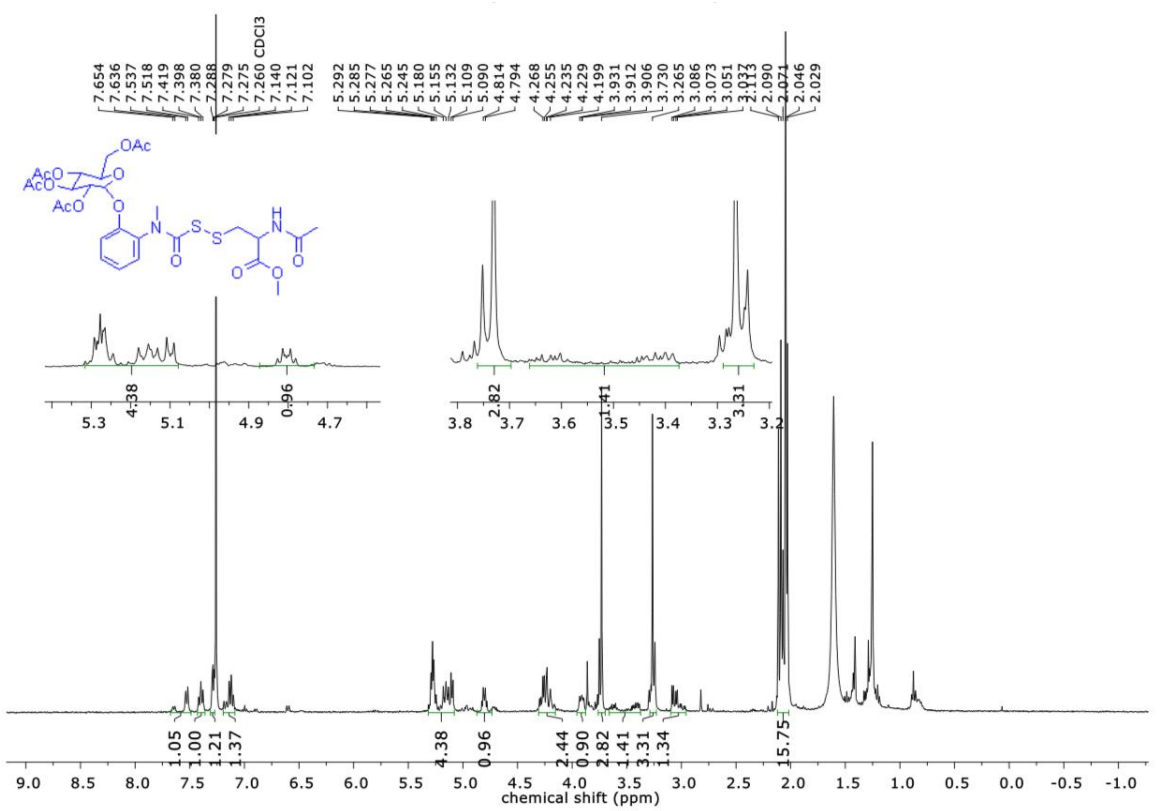
^{1}H and ^{13}C NMR spectra of **6**

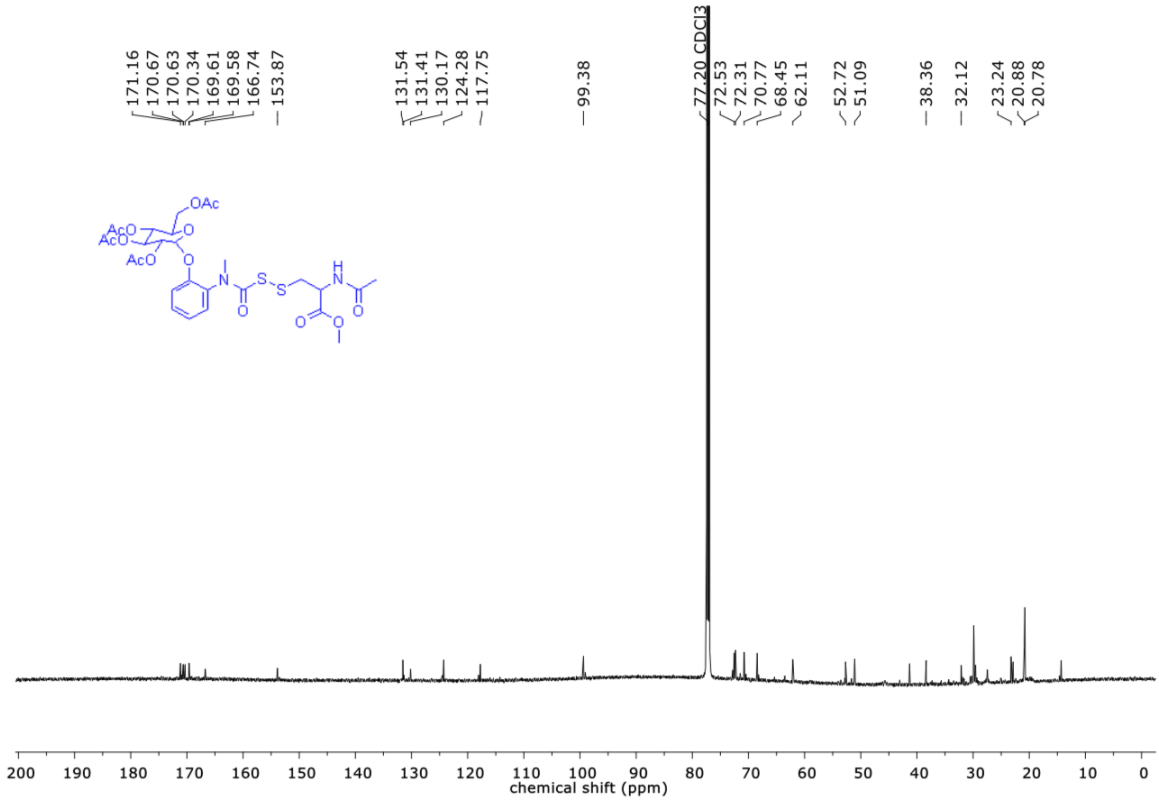


^{1}H and ^{13}C NMR spectra of **7**

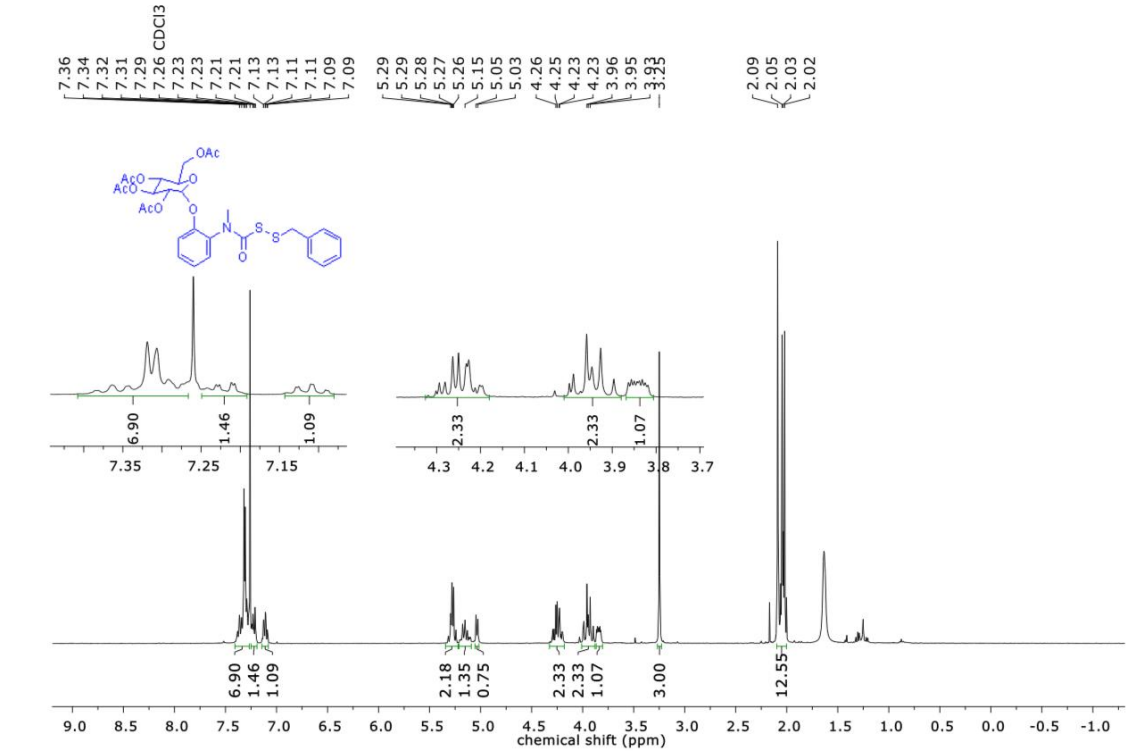


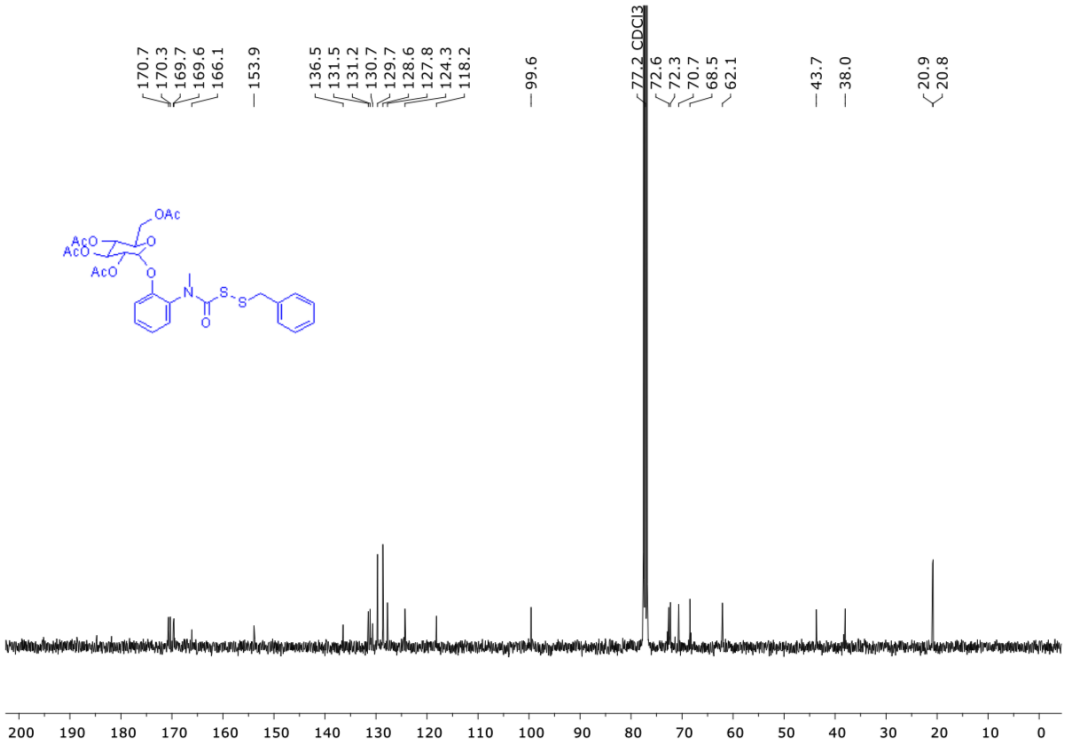
^{1}H and ^{13}C NMR spectra of **9a**



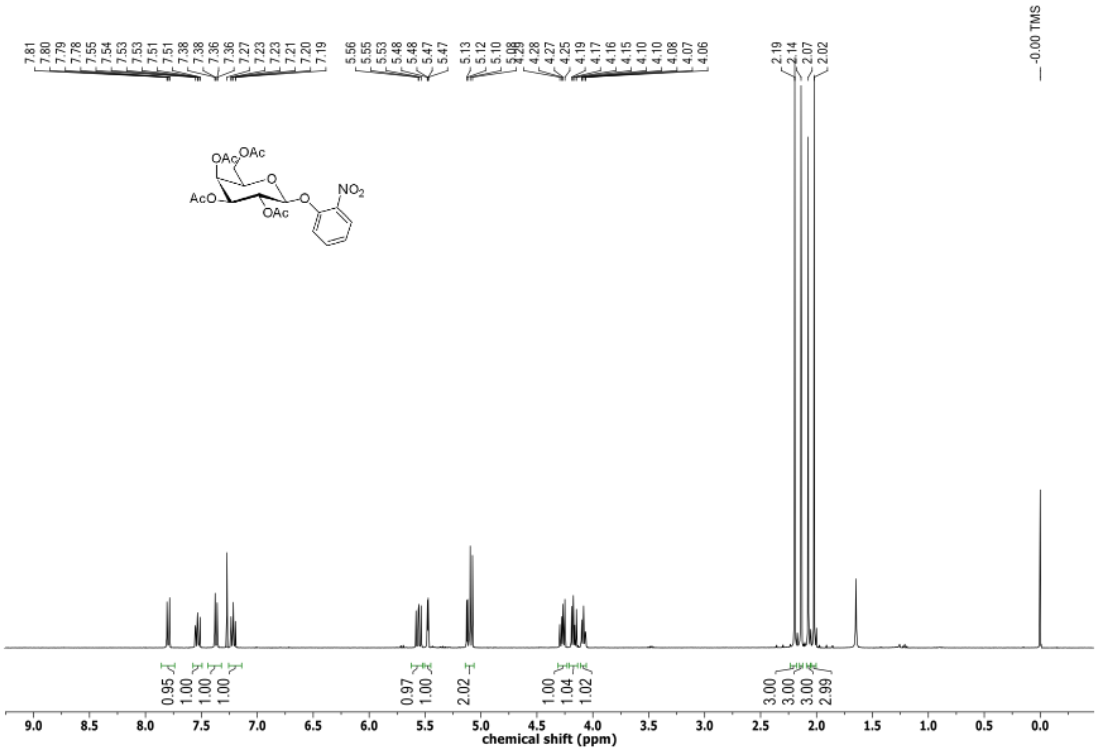


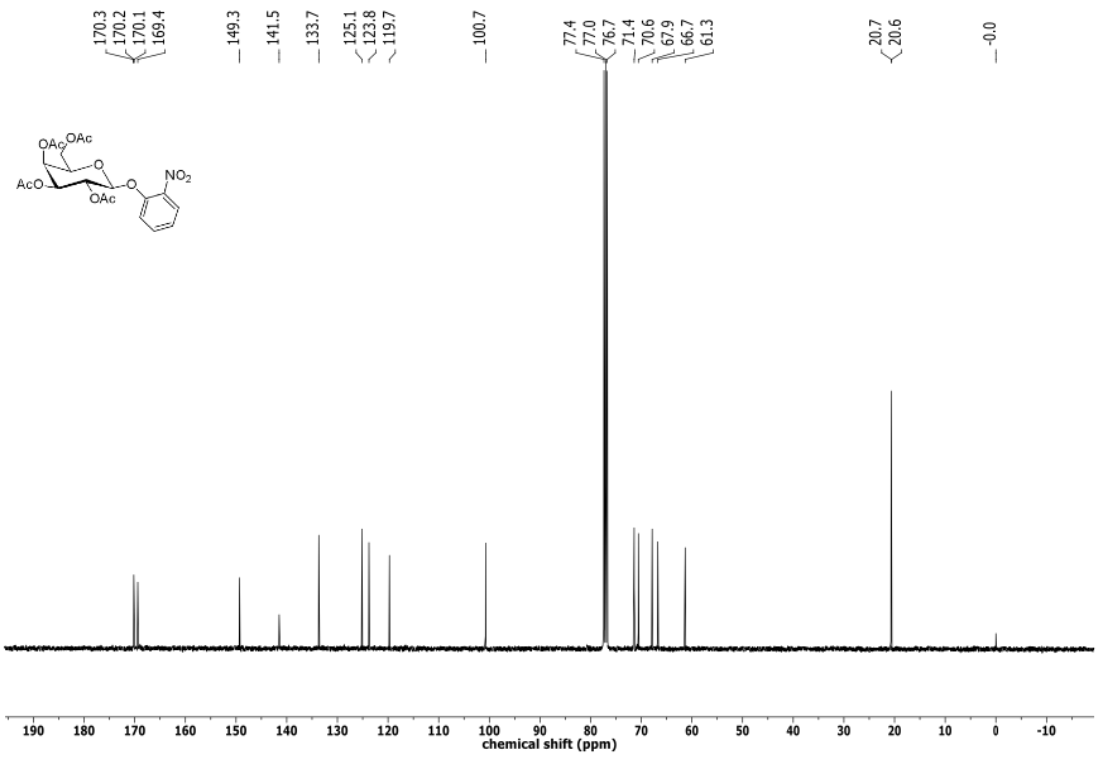
^{1}H and ^{13}C NMR spectra of **9b**



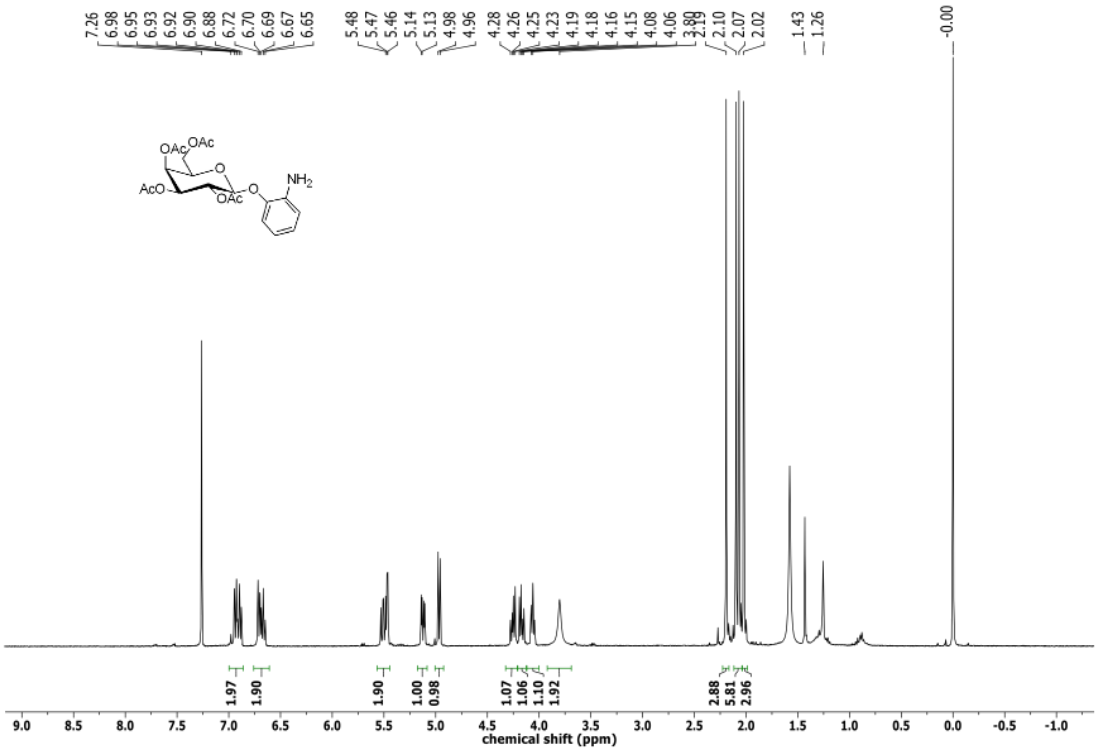


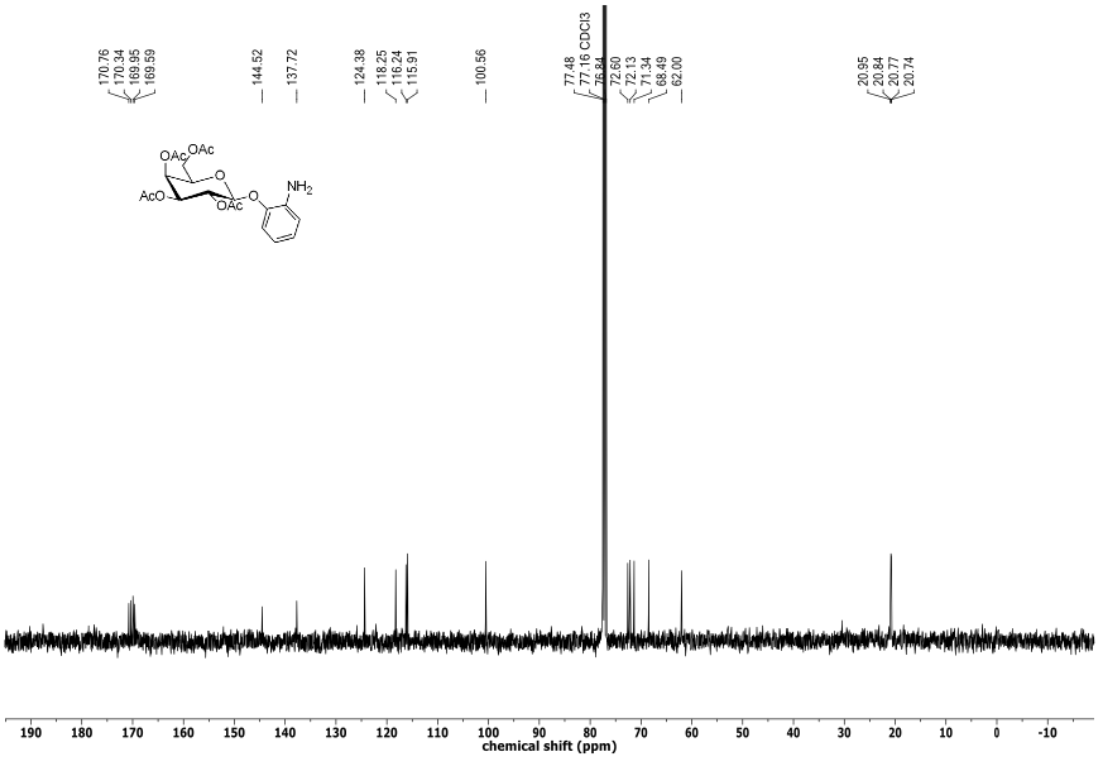
^{1}H and ^{13}C NMR spectra of **11**



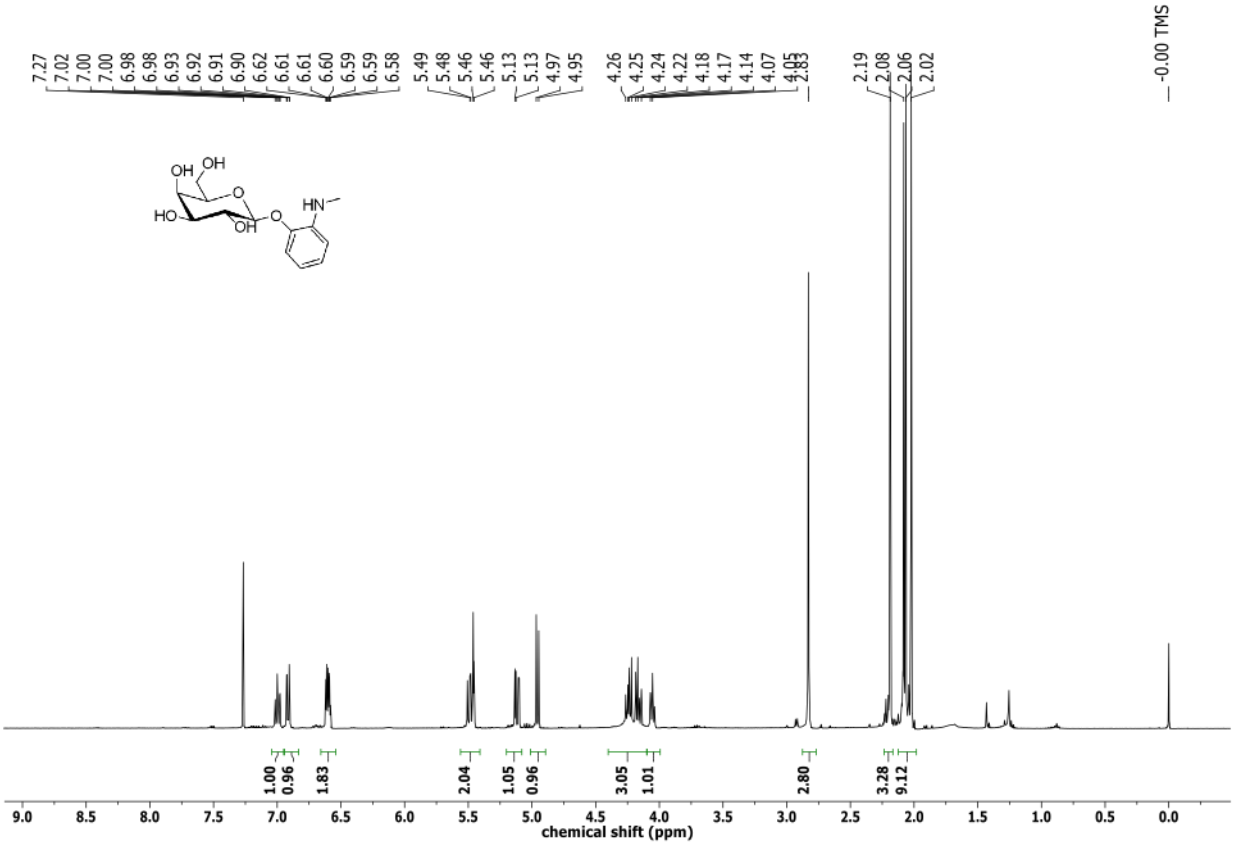


^{1}H and ^{13}C NMR spectra of **12**





^{1}H NMR spectra of **13**



^{1}H and ^{13}C NMR spectra of **14**