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SUPPORTING INFORMATION

Cancer cell death using metabolic glycan labelling techniques

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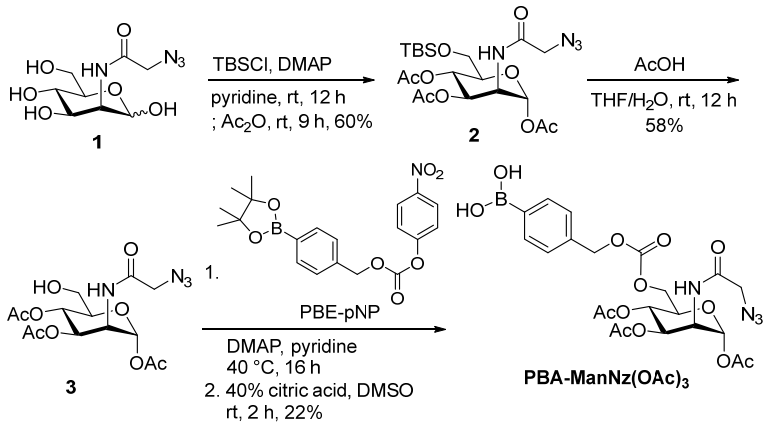
CONTENTS

Experimental details

Synthesis

General. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F254 glass plates (Merck Millipore). Compound spots were visualized by using a handheld UV lamp (254 nm) and/or by staining with 10 wt% phosphomolybdic acid in ethanol. Flash column chromatography was conducted by using silica gel 60 (230–400 mesh, Merck Millipore). NMR spectra were recorded on Bruker Avance lll HD 300 and Avance II 400 instruments. Coupling constants were reported in Hertz (Hz). High resolution mass spectra were obtained using an Agilent 6530 Accurate-Mass Q-TOF. Chemical reagents used in this study were purchased from Sigma-Aldrich, TCI, Acros Organics and Alfa Aesar.



Scheme S1. Synthesis of PBA-ManNAz(OAc)$_3$

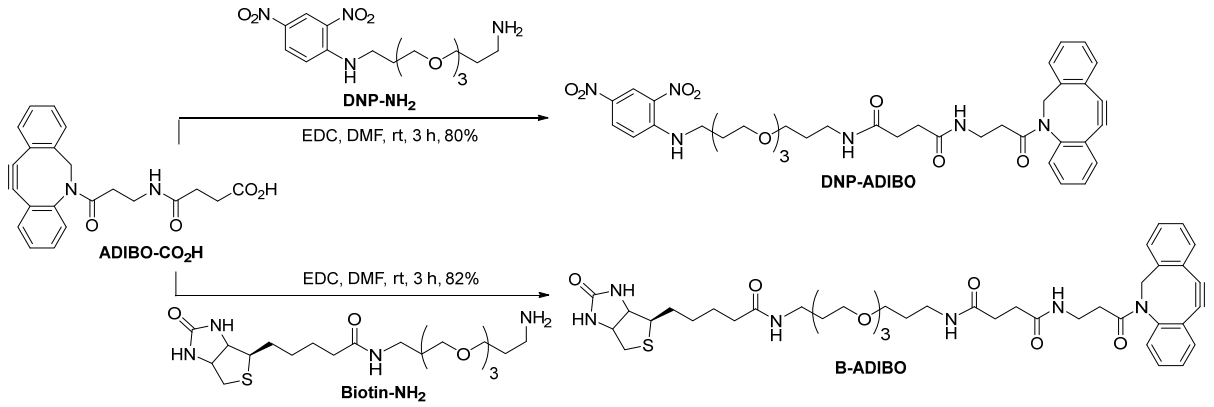
Compound 2. To a stirred solution of ManNAz[1] (**1**, 854 mg, 3.3 mmol) and DMAP (22 mg, 0.18 mmol) in anhydrous pyridine (5 mL) was added *t*-butyldimethylsilyl chloride (TBSCl, 540 mg, 3.6 mmol) in anhydrous pyridine (2 mL) at 0 ℃. The reaction mixture was warmed to room temperature. After stirring for 24 h, the mixture was cooled to 0 ℃ and acetic anhydride (9.4 mL, 99 mmol) was then added to the mixture. After stirring for 16 h at room temperature, the mixture was diluted with EtOAc, washed with brine and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (Hexane : EtOAc = 3:1) to give **2** as a white solid in 60% yield (982 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.53 (d, 1 H, *J* = 9.2 Hz), 5.97 (d, 1 H, *J* = 1.7 Hz), 5.28-5.25 (m, 2 H), 4.54 (d, 1 H, *J* = 9.7 Hz), 3.99 (d, 1 H, *J* = 16.7 Hz), 4.02 (d, 1 H, *J* = 16.7 Hz), 3.82-3.79 (m, 1 H), 3.69-3.57 (m, 2 H), 2.10 (s, 3 H), 1.98 (s, 3 H), 1.94 (s, 3 H), 0.86 (s, 9 H), 0.00 (d, 6 H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.0, 168.2, 166.8, 91.5, 72.8, 69.3, 65.2, 61.2, 53.5, 52.3, 49.3, 25.6, 20.8, 20.7, 20.6, 18.2, -5.56, -5.68; ESI-MS calcd for $C_{20}H_{34}N_4O_9Si$ $[M + Na]^+$ 525.2, found 525.5.

ManNAz(OAc)$_3$ (3).[2] To a stirred solution of **2** (503 mg, 1 mmol) in a mixture of THF and

H_2O (30 mL, 1:1) was added acetic acid (5.8 mL, 0.1 mmol) at room temperature. After stirring for 12 h at room temperature, the mixture was neutralized with saturated $NaHCO_3$. The mixture was diluted with EtOAc, washed with brine and water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (Hexane : EtOAc = 1:1) to give **ManNAz(OAc)$_3$** as a white solid in 58% yield (225 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (d, 1 H, *J* = 9.3 Hz), 6.06 (d, 1 H, *J* = 1.5 Hz), 5.40 (dd, 1 H, *J* = 10.3, 4.3 Hz), 5.18 (t, 1 H, *J* = 10.3 Hz), 4.66-4.63 (m, 1 H), 4.01 (s, 2 H), 3.84 (td, 1 H, *J* = 10.1, 2.2 Hz), 3.76-3.56 (m, 2 H), 3.03 (t, 1 H, *J* = 6.6 Hz), 2.19 (s, 3 H), 2.10 (s, 3 H), 2.02 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.1, 168.5, 167.3, 91.5, 72.5, 68.6, 65.4, 60.7, 52.2, 49.3, 20.9, 20.8, 20.7; ESI-MS calcd for $C_{14}H_{20}N_4O_9$ $[M + Na]^+$ 411.1, found 411.3.

PBA-ManNAz(OAc)$_3$. To a stirred solution of **ManNAz(OAc)$_3$** (388 mg, 1 mmol) and DMAP (178 mg, 1.46 mmol) in anhydrous pyridine (4 mL) was added PBE-pNP[3] (583 mg, 1.46 mmol) in anhydrous pyridine (2 mL) at room temperature. After stirring for 16 h at 40 °C, the mixture was cooled to room temperature, diluted with EtOAc, and washed with 1 M HCl and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (Hexane : EtOAc = 3:1) to give a phenylboronate ester-containing compound as a white solid in 40% yield (259 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, 2 H, *J* = 8.0 Hz), 7.38 (d, 2 H, *J* = 8.0 Hz), 6.74 (d, 1 H, *J* = 9.2 Hz), 6.05 (d, 1 H, *J* = 1.5 Hz), 5.35 (dd, 1 H, *J* = 10.4, 4.4 Hz), 5.22 (t, 1 H, *J* = 9.4 Hz), 5.18 (d, 2 H, *J* = 7.0 Hz), 4.65-4.61 (m, 1 H), 4.31-4.19 (m, 2 H), 4.09-4.05 (m, 1 H), 4.03-3.96 (m, 2 H), 2.17 (s, 3 H), 2.06 (s, 3 H), 2.01 (s, 3 H), 1.34 (s, 12 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.7, 168.2, 167.3, 154.7, 137.9, 135.1, 127.4, 91.3, 83.9, 75.0, 70.2, 70.0, 68.8, 65.6, 65.4, 52.2, 49.2, 24.9, 20.9, 20.8, 20.7; ESI-MS calcd for $C_{28}H_{37}BN_4O_{13}$ $[M + Na]^+$ 671.3, found 671.6.

To a stirred solution of a phenylboronate ester-containing compound (100 mg, 0.15 mmol) in DMSO (3 mL) was added saturated citric acid (2 mL) at room temperature. After stirring for 2 h, the mixture was diluted with EtOAc, washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC to give **PBA-ManNAz(OAc)$_3$** as a white solid in 54% yield (46 mg): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, 1 H, *J* = 9.1 Hz), 7.80 (d, 2 H, *J* = 7.7 Hz), 7.33 (d, 2 H, *J* = 7.7 Hz), 5.83 (s, 1 H), 5.25-5.11 (m, 4 H), 4.44-4.27 (m, 1 H), 4.26-4.08 (m, 3 H), 3.92 (s, 2 H), 2.15 (s, 3 H), 2.03 (s, 3 H), 1.94 (s, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 169.6, 168.5, 168.4, 137.0, 134.3, 127.1, 91.2, 69.7, 69.2, 68.5, 66.2, 65.4, 50.4, 48.7, 20.7, 20.6, 20.5; HR ESI-MS calcd for $C_{22}H_{27}BN_4O_{13}$ $[M + Na]^+$ 589.1560, found 589.1569.

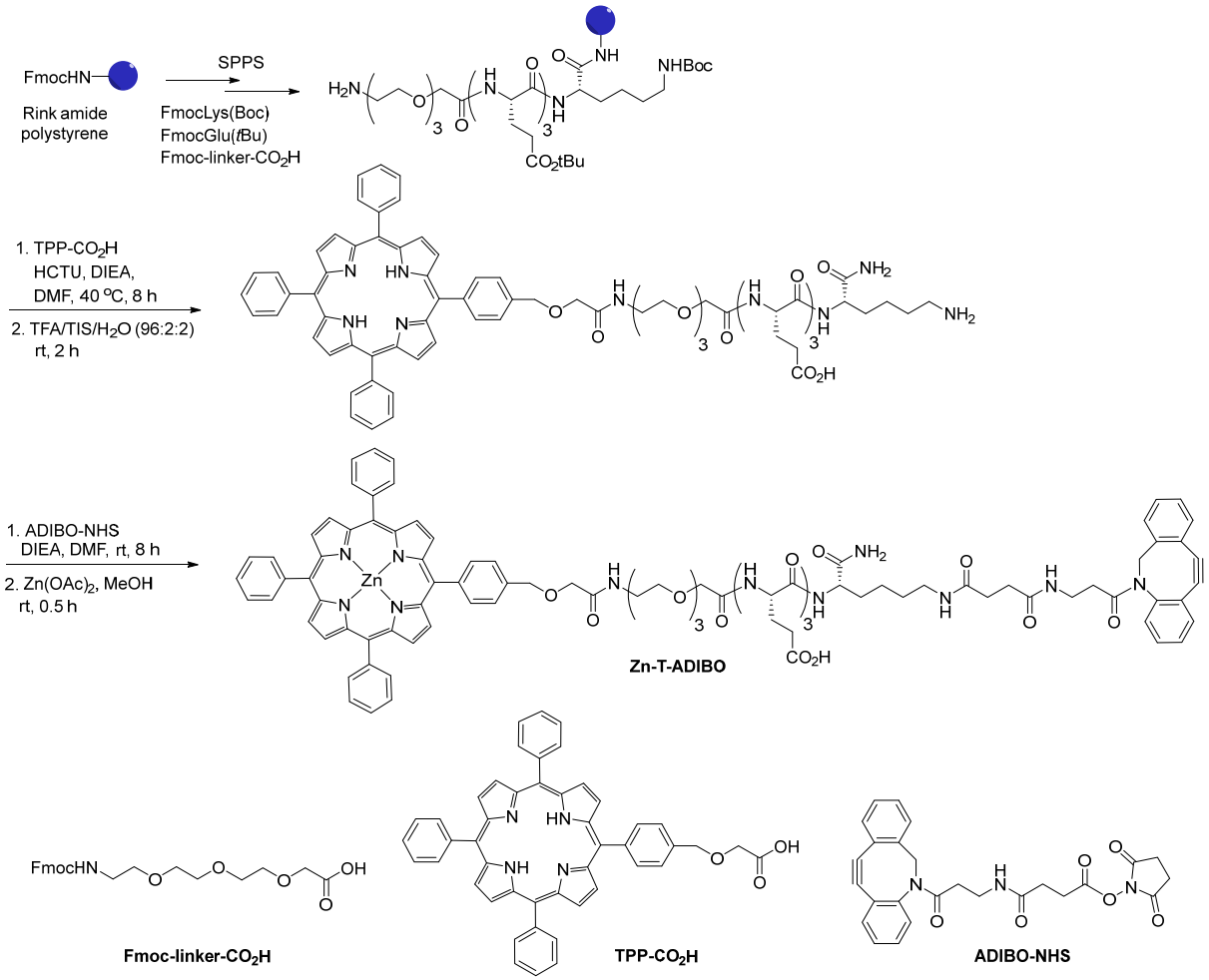


Scheme S2. Synthesis of DNP-ADIBO and B-ADIBO.

DNP-ADIBO. To a stirred solution of ADIBO-CO_2H (20 mg, 0.05 mmol, ref. 4) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide•HCl (EDC•HCl, 12 mg, 0.06 mmol) in DMF (1 mL) was added DNP-NH_2 (23 mg, 0.43 mmol, ref. 5) at room temperature. After stirring for 3 h at room temperature, the mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2 : MeOH = 20:1) to afford **DNP-ADIBO** as a yellowish solid in 80% yield (29 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (d, 1 H, *J* = 2.6 Hz), 8.91 (s, 1 H), 8.29 (dd, 1 H, *J* = 9.3, 2.4 Hz), 7.70 (d, 1 H, *J* = 7.9 Hz) 7.42-7.30 (m, 7 H), 6.99 (d, 1 H, *J* = 9.4 Hz), 5.17 (d, 1 H, *J* = 14.6 Hz), 3.73-3.60 (m, 8 H), 3.59-3.53 (m, 6 H), 3.35–3.32 (m, 2 H), 3.19-3.22 (m, 1 H), 2.53-2.36 (m, 5 H), 2.09-2.00 (m, 3 H), 1.80-1.75 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 172.0, 171.9, 148.4, 147.9, 135.8, 132.1, 130.3, 129.1, 128.6, 127.9,127.2, 125.6, 124.3, 122.8, 114.9, 113.8, 70.6, 70.5, 70.5, 70.1, 69.7, 69.2, 55.5, 41.9, 37.9, 35.4, 34.5, 31.4, 29.7, 28.9, 28.6; HR ESI-MS calcd for $C_{38}H_{44}N_6O_{10}$ $[M + H]^+$ 745.3191, found 745.3191.

B-ADIBO. To a stirred solution of ADIBO-CO_2H (20 mg, 0.05 mmol) and EDC•HCl (12 mg, 0.06 mmol) in DMF (1 mL) was added Biotin-NH_2 (26 mg, 0.06 mmol, ref. 6) at room temperature. After stirring for 3 h at room temperature, the mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2 : MeOH = 10:1) to afford **B-ADIBO** as a yellowish solid in 82% yield (32 mg): ^{1}H NMR (400 MHz, CD_3OD) δ 7.69 (d, 1 H, *J* = 7.5 Hz), 7.52-7.44 (m, 3 H), 7.42-7.32 (m, 2 H), 7.29-7.27 (m, 1 H), 7.17-7.14 (m, 1 H), 5.18 (d, 1 H, *J* = 14.3 Hz), 4.51 (dd, 1 H, *J* = 7.5, 4.5 Hz), 4.32 (dd, 1 H, *J* = 7.5, 4.5 Hz), 3.75 (d, 1 H, *J* = 14.3 Hz), 3.65-3.50 (m, 16 H), 3.28-3.22 (m, 6 H), 3.16-3.15 (m, 1 H), 2.95 (dd, 1 H, *J* = 12.0, 4.5 Hz), 2.73 (d, 1 H, *J* = 12.0), 2.47-2.43 (m, 2 H), 2.36-2.31 (m, 4 H), 2.22-2.18 (m, 3 H), 2.10-2.04 (m, 1 H), 1.79-1.60 (m, 10 H), 1,46-1.42 (m, 2 H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.5, 172.9, 171.7, 164.7, 151.1, 148.0, 132.0, 129.1, 128.6, 128.3, 127.8, 127.5, 126.7, 125.1, 122.9,

122.2, 114.2, 107.4, 70.1, 69.8, 68.4, 61.9, 60.2, 55.6, 55.2, 39.6, 36.4, 35.4, 35.3, 30.8, 28.9, 28.4, 28.1, 25.5; HR ESI-MS calcd for $C_{42}H_{56}N_6O_8S$ $[M + H]^+$ 805.3953, found 805.3953.



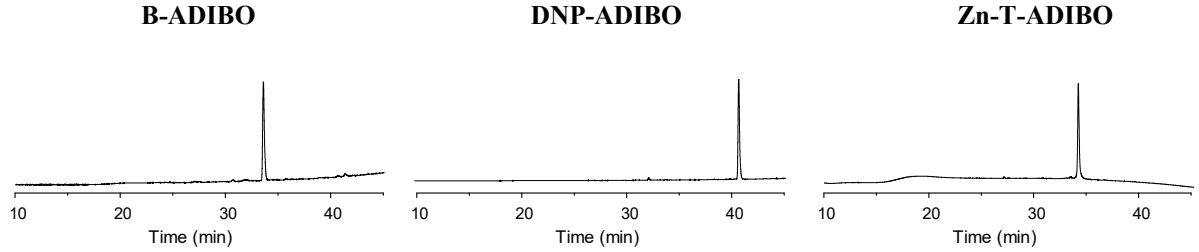
Scheme S3. Synthesis of Zn-T-ADIBO.

Zn-T-ADIBO. A peptide was manually synthesized by using the Fmoc/tBu strategy on Rink amide polystyrene resins (0.5 mmol/g). Fmoc amino acids (3.0 equiv) and Fmoc-linker-CO_2H (3.0 equiv) were coupled on resins (5.0 mmol) in the presence of O-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HCTU, 2.8 equiv) and DIEA (6.0 equiv). After each coupling reaction, the Fmoc group was removed by treatment with 20% piperidine in DMF for 15 min and the resin was washed with DMF and CH_2Cl_2 several times (repeat this step twice). TPP-CO_2H (2.0 equiv, ref. 7) was coupled on resins (5.0 mmol) in the presence of HCTU (1.8 equiv) and DIEA (4.0 equiv) for 8 h at 40 °C. The TPP-appended peptide was cleaved from the solid support by treatment with TFA/triisopropylsilane (TIS)/H_2O (96:2:2, v/v/v) for 2 h. The TPP-appended peptide was purified by preparative RP-HPLC (250 x 40 mm) using a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA)

over 60 min. To a stirred solution of ADIBO-NHS (10 mg, 0.025 mmol) and TPP-appended peptide (6 mg, 0.01 mmol) in DMF (1 mL) was added DIEA (10 μL, 0.025 mmol) at room temperature. After stirring for 8 h at room temperature, the mixture was diluted with MeOH and $Zn(OAc)_2$ was added to the mixture. After stirring for 0.5 h, **Zn-T-ADIBO** was purified by preparative RP-HPLC (250 x 40 mm) using a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 60 min: MALDI-TOF MS calcd for $C_{95}H_{93}N_{13}O_{19}Zn$ $[M + H]^+$ 1784, found 1784.

<HPLC profiles>

Analytic RP-HPLC (C18 column, 250 x 4.6 mm) with a gradient of 5-100% CH_3CN in water (0.1% TFA) over 45 min (a flow rate; 1 mL/min, detection at 254 nm).



Removal of a PBA group from PBA-ManNAz(OAc)$_3$ by H_2O_2. A mixture of PBA-ManNAz(OAc)$_3$ and H_2O_2 in H_2O/MeOH (2:1) was stirred for 2 h at room temperature. The mixture was diluted with CH_2Cl_2. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. NMR spectra of crude products were taken by using 400 MHz ^{1}H NMR spectrometer.

Cell study

Cell culture. HT29 (human colon adenocarcinoma), HCT116 (human colorectal carcinoma) and MCF7 (human breast adenocarcinoma) cells were cultured in RPMI or DMEM (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 50 units/mL penicillin and 50 units/mL streptomycin. NK92-CD16 cells were cultured in alpha minimum essential medium (alpha MEM) supplemented with 25% FBS and recombinant human interleukin-2 (200 U/mL). Cells were maintained at 37 ^oC under a humidified atmosphere containing 5% CO_2.

Fluorescence confocal microscopy image of cells. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS (Dulbecco's phosphate-buffered saline), cells were incubated with 200 μM PBA-ManNAz(OAc)$_3$ for 24 h. After washing with DPBS, cells were incubated with 100 μM B-ADIBO for 1 h. After washing with DPBS, cells were incubated with Cy3-strepavidin for 1 h. After washing with DPBS, cell images were obtained by using confocal fluorescence microscopy (LSM800).

Lactate dehydrogenase (LDH) assay. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were incubated with 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. After washing with DPBS, cells were incubated with 100 μM DNP-ADIBO for 1 h. After washing with DPBS, NK92-CD16 cells (E) were distributed in triplicate at E:T ratios of 0.1:1, 0.5:1, 1:1, and 2:1 in the presence and absence of 5 μg/mL DNP antibody. After incubation for 4 h, cells were centrifuged and the supernatant was collected. A mixture of WST-1 (water soluble tetrazolium salts-1, 15 μM), NAD (nicotinamide adenine dinucleotide, 14 μM) and lactate (35 μM) was added to the supernatant and incubated for 1 h. Triton X-100 was used as a control for complete permeabilization of cells. The absorbance at 490 nm was measured by using an Infinite 200 PRO multimode microplate reader (Tecan). Cell death (%) was calculated by using the following equation

$$\frac{(Real\ sample - untreated)}{(Complete\ permeabilization - untreated)}\ x\ 100$$

Granzyme B activity assay. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were incubated with 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. After washing with DPBS, treated cells were incubated with 100 μM DNP-ADIBO for 1 h. NK92-CD16 cells were distributed in triplicate at E:T ratios of 0.1:1, 0.5:1, 1:1, and 2:1 in the presence and absence of 5 μg/mL DNP antibody. After incubation for 4 h, cells were centrifuged and the supernatant was collected. Granzyme B activity was measured by using a granzyme B activity assay kit (Abcam) according to the manufacture's protocol. Briefly, cells were homogenized and the supernatant was used to determine the activity of granzyme B by using a microplate reader at Ex/Em = 380/500 nm (Tecan).

Perforin ELISA. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were incubated with 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. After washing with DPBS, treated cells were incubated with 100 μM DNP-ADIBO for 1 h. NK92-CD16 cells were distributed in triplicate at E:T ratios of 0.1:1, 0.5:1, 1:1, and 2:1 in the presence and absence of 5 μg/mL DNP antibody. After incubation for 4 h, cells were centrifuged and the supernatant was collected. The level of perforin in the supernatant was measured by using a Perforin ELISA kit (Abcam) according to the manufacture's protocol. Briefly, 100 μL of the cell culture supernatant was added to the antibody-coated microplate strips and incubated for 2 h at room temperature. The cells were washed with the assay buffer and 200 μL of horseradish peroxidase-conjugated antibody was added to each well and incubated for additional 2 h. The wells were washed and incubated with chromogen tetramethylbenzidine for 30 min. The reaction was stopped by addition of 2 N sulfuric acid and the absorbance at 450 nm was measured by using an Infinite 200 PRO multimode microplate reader (Tecan).

Photocytotoxicity. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were incubated with 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. After washing with DPBS, treated cells were incubated with 100 μM Zn-T-ADIBO for 1 h. After washing with DPBS, cells were irradiated by means of a light-emitting diode (LED) device for 15 min on ice. After incubation for 24 h without light irradiation, cells were centrifuged and the supernatant was collected. A mixture of WST-1 (water soluble tetrazolium salts-1, 15 μM), NAD (nicotinamide adenine dinucleotide, 14 μM) and lactate (35 μM) was added to the supernatant and incubated for 1 h. Triton X-100 was used as a control for complete permeabilization of cells. The absorbance at 490 nm was measured by using an Infinite 200 PRO multimode microplate reader.

Detection of singlet oxygen. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were incubated with 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. After washing with DPB, treated cells were incubated with 100 μM Zn-T-ADIBO for 1 h. After washing with DPBS, cells were treated with 25 μM singlet oxygen sensor green (SOSG) for 30 min and then irradiated by means of a light-emitting diode (LED) device for 15 min on ice. After incubation for 30 min without light irradiation, cell images were obtained by using confocal fluorescence microscopy (LSM800). Hoechst 33342 was used to stain nuclei of cells.

Detection of chromatin condensation. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were incubated with 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. After washing, treated cells were incubated with 100 μM Zn-T-ADIBO for 1 h. After washing with DPBS, cells were irradiated by means of a light-emitting diode (LED) device for 15 min on ice. After incubation for 12 h, cells were stained with Hoechst 33342 for 5 min and cell images were obtained by using confocal fluorescence microscopy (LSM800).

Measurement of caspase activity. HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were incubated with 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. After washing with DPBS, treated cells were incubated with 100 μM Zn-T-ADIBO for 1 h. After washing with DPBS, cells were irradiated by means of a light-emitting diode (LED) device for 15 min on ice. After incubation for 24 h without light irradiation, cells were lysed in a buffer containing 50 mM HEPES at pH 7.4, 5 mM CHAPS, and 5 mM DTT. Cell lysates were placed into the appropriate wells of 96-well plates. Assay buffer containing 20 mM HEPES at pH 7.4, 0.01% CHAPS, 5 mM DTT and 2 mM EDTA was added to each well. Caspase activity was determined by adding 200 μM Ac-DEVD-pNA (Sigma-Aldrich, USA) to each well. The enzyme-catalyzed release of pNA was monitored at 405 nm using a microplate reader.

Flow cytometry. For analysis of apoptosis, HT29, HCT116 and MCF7 cells were incubated with 50 μM H_2O_2 for 1 h. After washing, cells were incubated with 200 μM PBA-$ManNAz(OAc)_3$ for 24 h. After washing, treated cells were incubated with 100 μM Zn-T-ADIBO for 1 h. After washing, cells were irradiated by means of a light-emitting diode (LED) device for 15 min on ice. After incubation for 24 h without light irradiation, cells were washed with PBS. The cells were trypsinized with 0.5 mL of trypsin-EDTA for 5-10 min at 37 ^{o}C and collected by centrifugation. The cells were stained with the FITC-annexin V apoptosis detection kit according to the manufacturer's protocol. Flow cytometry was performed using a BD FACSVerse™ instrument (BD Biosciences) and the data were analyzed using a FlowJo™ software (BD Biosciences).

Quantitation of fluorescence intensity in cells. Fluorescence intensities in cells were quantified for each image using the mean region of interest (ROI) tool with the ZEN software. Specifically, a constant circular ROI was chosen to encompass cells of interest and the same ROI area size was used for background subtraction. All confocal microscopy images were representative of at least three independent experiments.

Supplementary References

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Supplementary Figures and Figure Legends

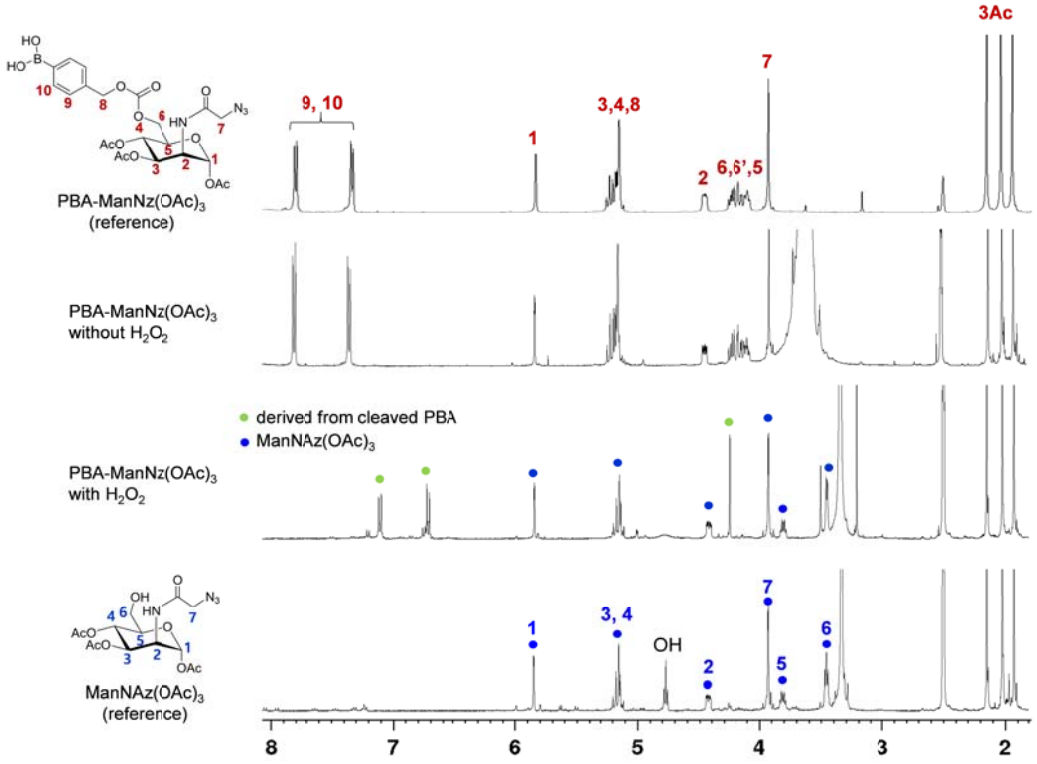


Figure S1. Removal of a PBA group from PBA-ManNAz(OAc)$_3$ by H_2O_2. Reaction mixtures of PBA-ManNAz(OAc)$_3$ and H_2O_2 in H_2O-MeOH (2:1) for 2 h at room temperature were analyzed by ^{1}H NMR spectrometer. As a control, solutions of PBA-ManNAz(OAc)$_3$ in H_2O-MeOH (2:1) for 2 h at room temperature were analyzed (NMR solvent: DMSO-d_6).

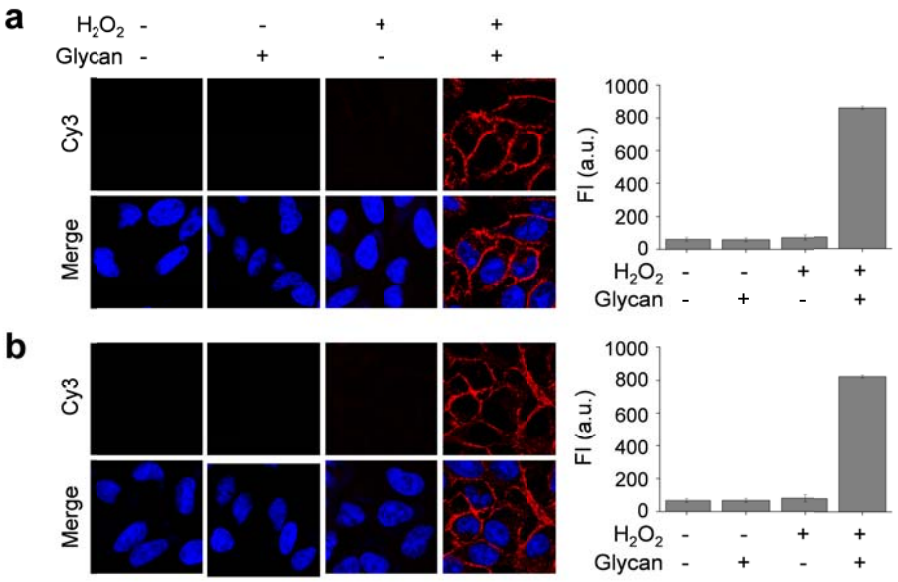


Figure S2. (a) HCT116 and (b) MCF7 cells were treated with 50 μM H_2O_2 for 1 h and then 200 μM PBA-ManNAz$(OAc)_3$ for 24 h. Treated cells were exposed to 100 μM B-ADIBO, followed by incubation with Cy3-streptavidin. Bottom images: merged images of Hoechst 33342 and Cy3 fluorescence (scale bar = 10 μm). Graphs show fluorescence intensity (FI) of Cy**3** in cells (mean ± s.d., n = 3).

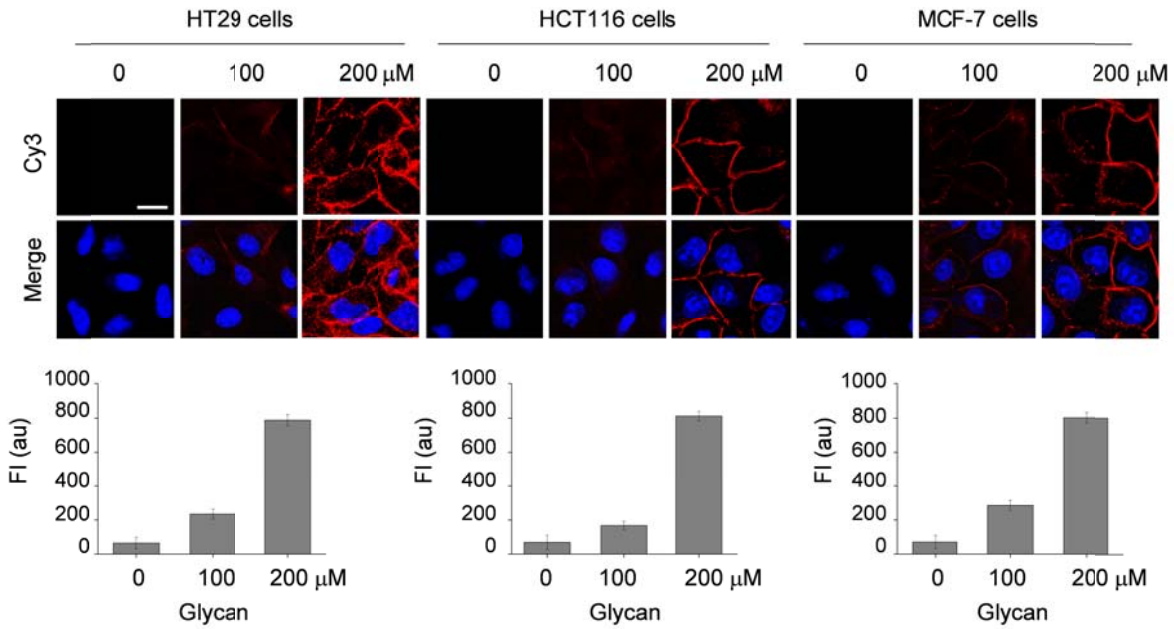


Figure S3. Cancer cells were incubated with 50 μM H_2O_2 for 1 h. After washing with DPBS, cells were treated with indicated concentrations of PBA-ManNAz$(OAc)_3$ (Glycan) for 24 h. After washing, cells were exposed to 100 μM B-ADIBO for 1 h followed by incubation with Cy3-streptavidin for 1 h. Cell images were obtained by using confocal fluorescence microscopy. Hoechst 33342 was used for staining nuclei (scale bar = 10 μm). Graphs show fluorescence intensity of Cy3 in cells (mean ± s.d., n = 3).

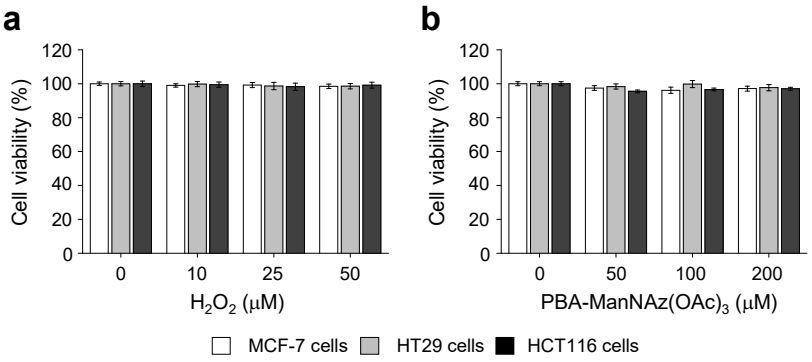


Figure S4. Effect of H_2O_2 and PBA-ManNAz(OAc)$_3$ on cell viability. Cancer cells were incubated with indicated concentrations of (a) H_2O_2 for 1 h and (b) PBA-ManNAz(OAc)$_3$ for 24 h. Cell viability was measured by means of an MTT assay (mean ± s.d., n = 3).

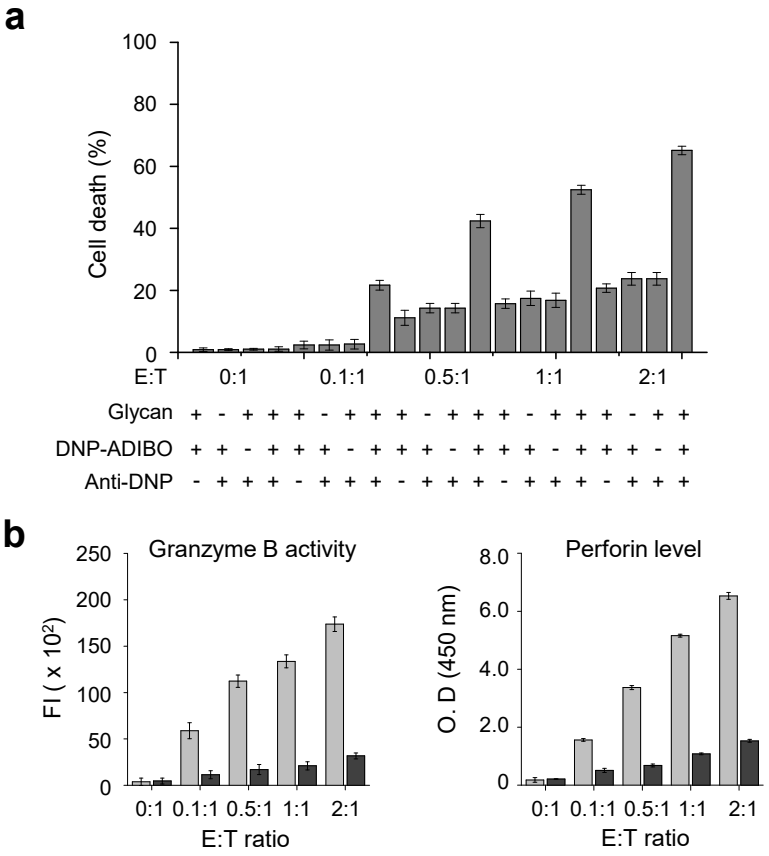


Figure S5. (a) HCT116 cells, pretreated with 50 μM H_2O_2 for 1 h, were sequentially incubated with 200 μM PBA-ManNAz(OAc)$_3$ (Glycan) for 24 h and 100 μM DNP-ADIBO for 1 h. The treated cells were incubated with 5 μg/mL of DNP antibody for 1 h. As controls, cells were incubated under conditions lacking one of the three components (PBA-ManNAz(OAc)$_3$, DNP-ADIBO and DNP antibody). The treated target cells (T) were co-cultured for 4 h with NK92-CD16 cells (E) at various E:T ratios. Cancer cell death was determined using the LDH assay (mean ± s.d., n = 3). (b) HCT116 cells, pretreated with 50 μM H_2O_2 for 1 h, were sequentially incubated with 200 μM PBA-ManNAz(OAc)$_3$ for 24 h and 100 μM DNP-ADIBO for 1 h. Treated cells were incubated for 1 h without (black bar) or with (grey bar) 5 μg/mL of DNP antibody. The treated cells were co-cultured for 4 h with NK92-CD16 cells at various E:T ratios. (Left) Granzyme B activity and (right) the level of perforin were determined using granzyme B activity assay kit and ELISA, respectively (mean ± s.d., n = 3).

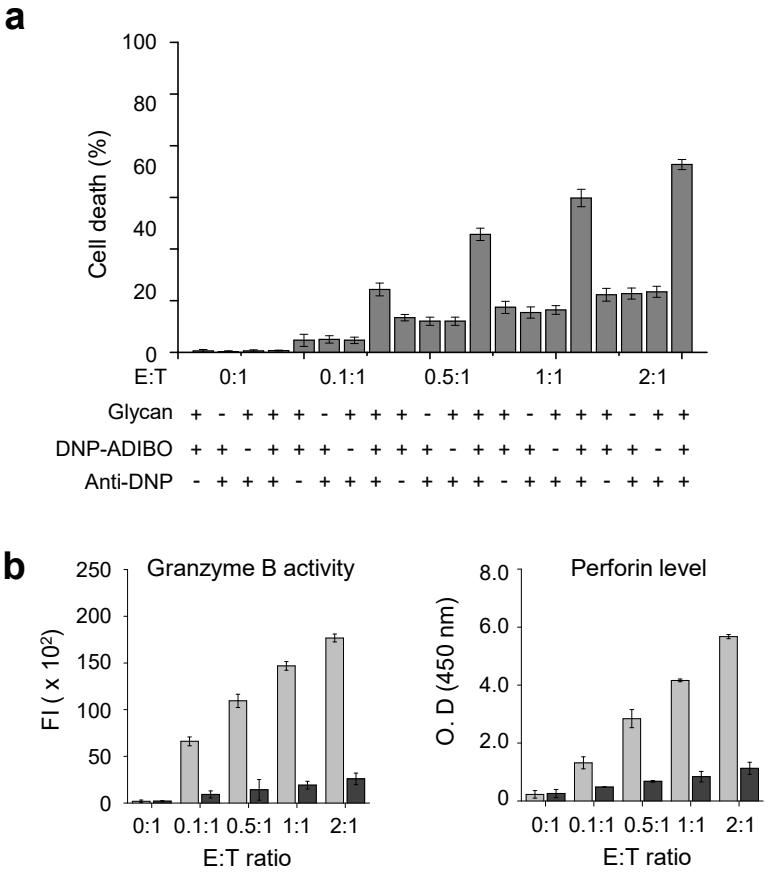


Figure S6. (a) MCF7 cells, pretreated with 50 μM H_2O_2 for 1 h, were sequentially incubated with 200 μM PBA-ManNAz(OAc)$_3$ (Glycan) for 24 h and 100 μM DNP-ADIBO for 1 h. The treated cells were incubated with 5 μg/mL of DNP antibody for 1 h. As controls, cells were incubated under conditions lacking one of the three components (PBA-ManNAz(OAc)$_3$, DNP-ADIBO and DNP antibody). The treated target cells (T) were co-cultured for 4 h with NK92-CD16 cells (E) at various E:T ratios. Cancer cell death was determined using the LDH assay (mean ± s.d., n = 3). (b) MCF7 cells, pretreated with 50 μM H_2O_2 for 1 h, were sequentially incubated with 200 μM PBA-ManNAz(OAc)$_3$ for 24 h and 100 μM DNP-ADIBO for 1 h. Treated cells were incubated for 1 h without (black bar) or with (grey bar) 5 μg/mL of DNP antibody. The treated cells were co-cultured for 4 h with NK92-CD16 cells at various E:T ratios. (Left) Granzyme B activity and (right) the level of perforin were determined using granzyme B activity assay kit and ELISA, respectively (mean ± s.d., n = 3).

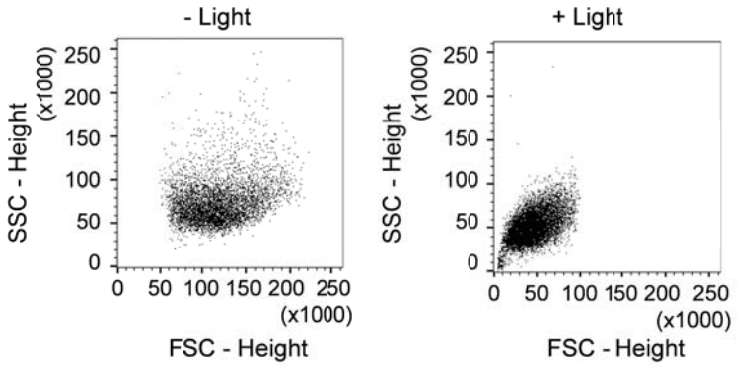


Figure S7. HT29 cells, pretreated with H_2O_2, were sequentially incubated with PBA-ManNAz$(OAc)_3$ (Glycan) and Zn-T-ADIBO, followed by exposure to light. Treated cells were incubated for additional 24 h. Shown is flow cytometry of cell size.

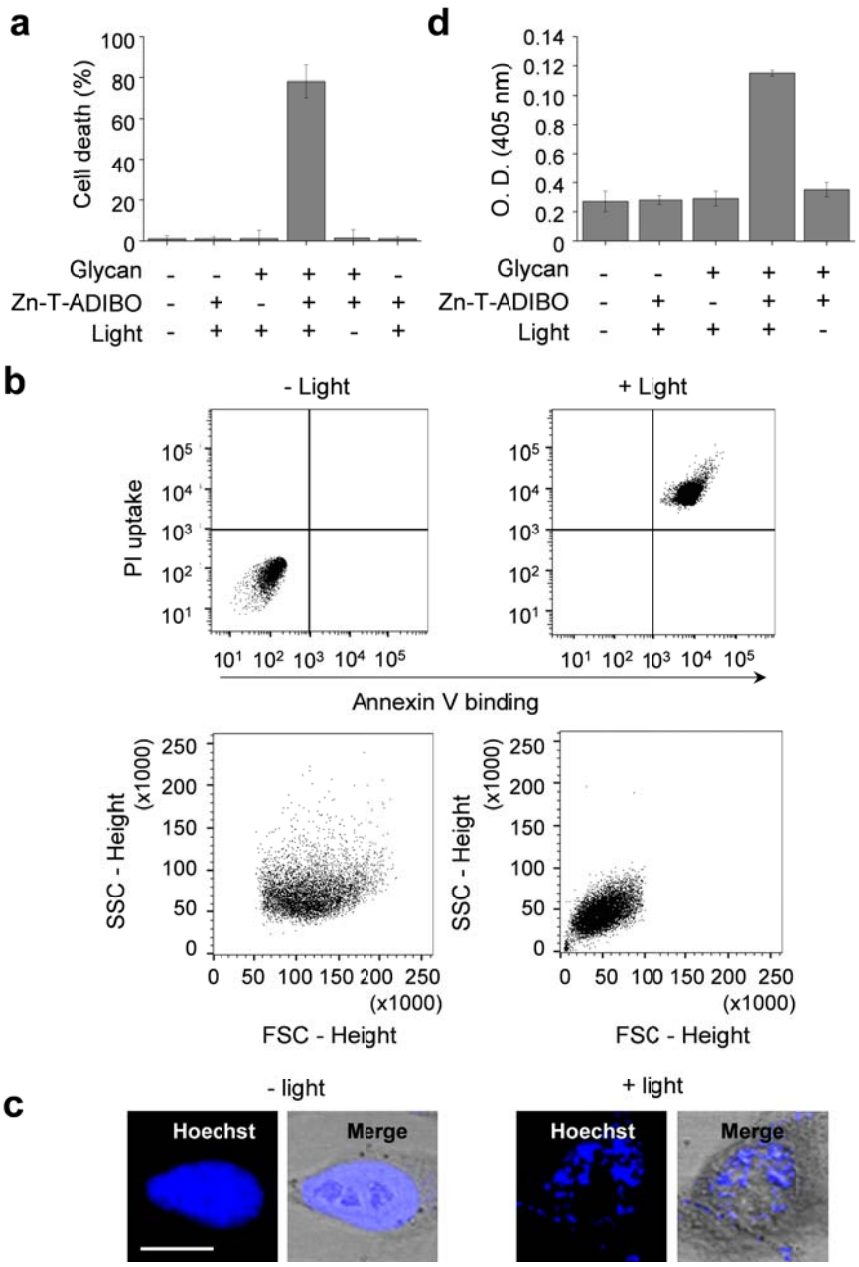


Figure S8. HCT116 cells, pretreated with H_2O_2, were sequentially incubated with PBA-ManNAz(OAc)$_3$ (Glycan) and Zn-T-ADIBO, followed by exposure to light. As controls, cells were incubated under conditions lacking one of the two components (Glycan and Zn-T-ADIBO) or light illumination. In addition, cells incubated with Zn-T-ADIBO alone were exposed to light. Treated cells were incubated for additional 24 h. (a) Cell death was determined using LDH assays (mean ± s. d., n = 3). (b) (Upper) Flow cytometry of FITC-annexin V and PI and (lower) cell size. (c) Sequentially treated cells were (right) irradiated with light for 15 min and (left) not irradiated. Merged images of Hoechst 33342 and differential interference contrast (DIC) are shown (scale bar = 10 μm). (d) Caspase activities of treated cell lysates were determined using Ac-DEVD-pNA (mean ± s. d., n = 3).

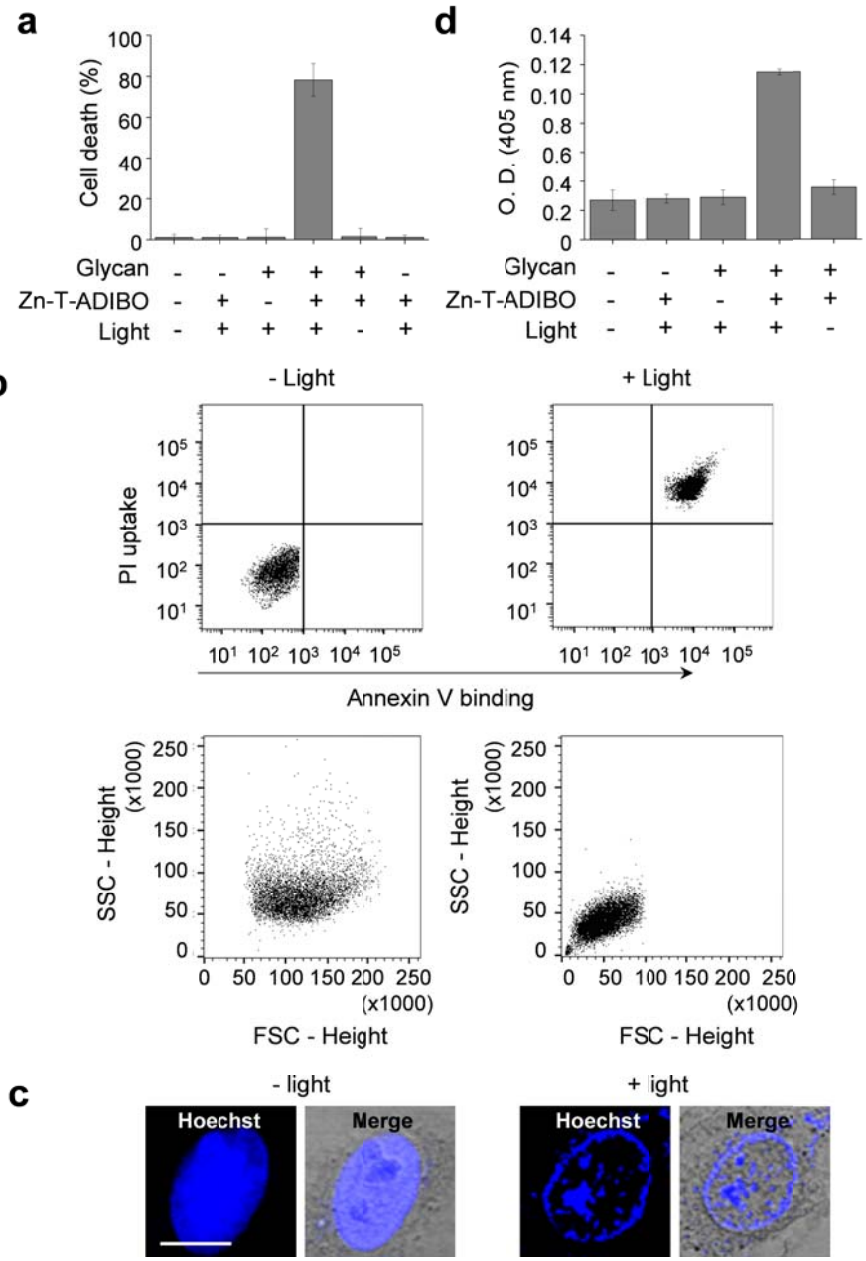


Figure S9. MCF7 cells, pretreated with H_2O_2, were sequentially incubated with PBA-ManNAz$(OAc)_3$ (Glycan) and Zn-T-ADIBO, followed by exposure to light. As controls, cells were incubated under conditions lacking one of the two components (Glycan and Zn-T-ADIBO) or light illumination. In addition, cells incubated with Zn-T-ADIBO alone were exposed to light. Treated cells were incubated for additional 24 h. (a) Cell death was determined using LDH assays (mean ± s. d., n = 3). (b) (Upper) Flow cytometry of FITC-annexin V and PI and (lower) cell size. (c) Sequentially treated cells were (right) irradiated with light for 15 min and (left) not irradiated. Merged images of Hoechst 33342 and differential interference contrast (DIC) are shown (scale bar = 10 μm). (d) Caspase activities of treated cell lysates were determined using Ac-DEVD-pNA (mean ± s. d., n = 3).

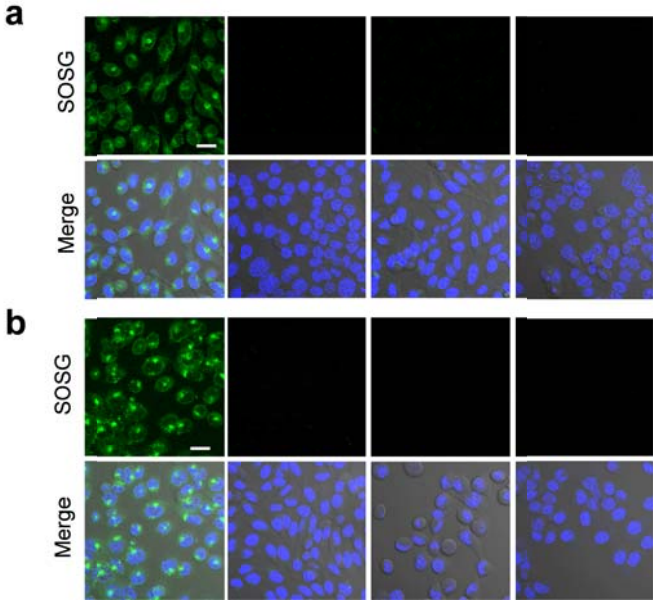


Figure S10. (a) HCT116 and (b) MCF7 cells, pretreated with H_2O_2, were sequentially incubated with PBA-ManNAz$(OAc)_3$ (Glycan), Zn-T-ADIBO, and SOSG. Treated cells were exposed to light. Hoechst 33342 was used for staining nuclei. Bottom images: merged images of SOSG and Hoechst 33342 fluorescence (scale bar = 10 μm).

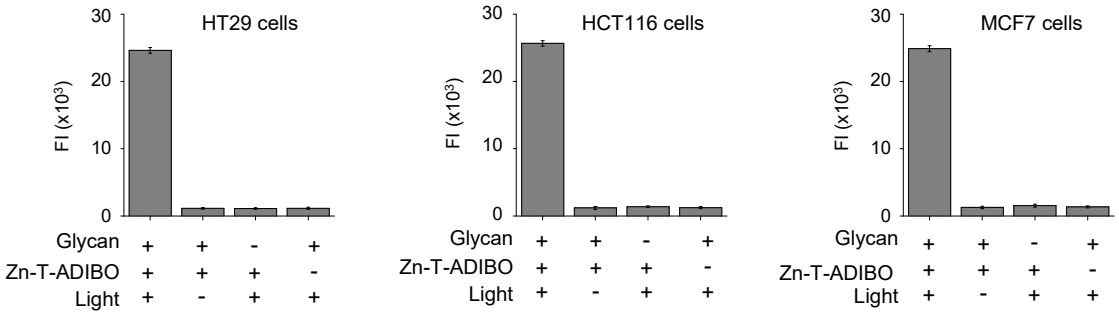
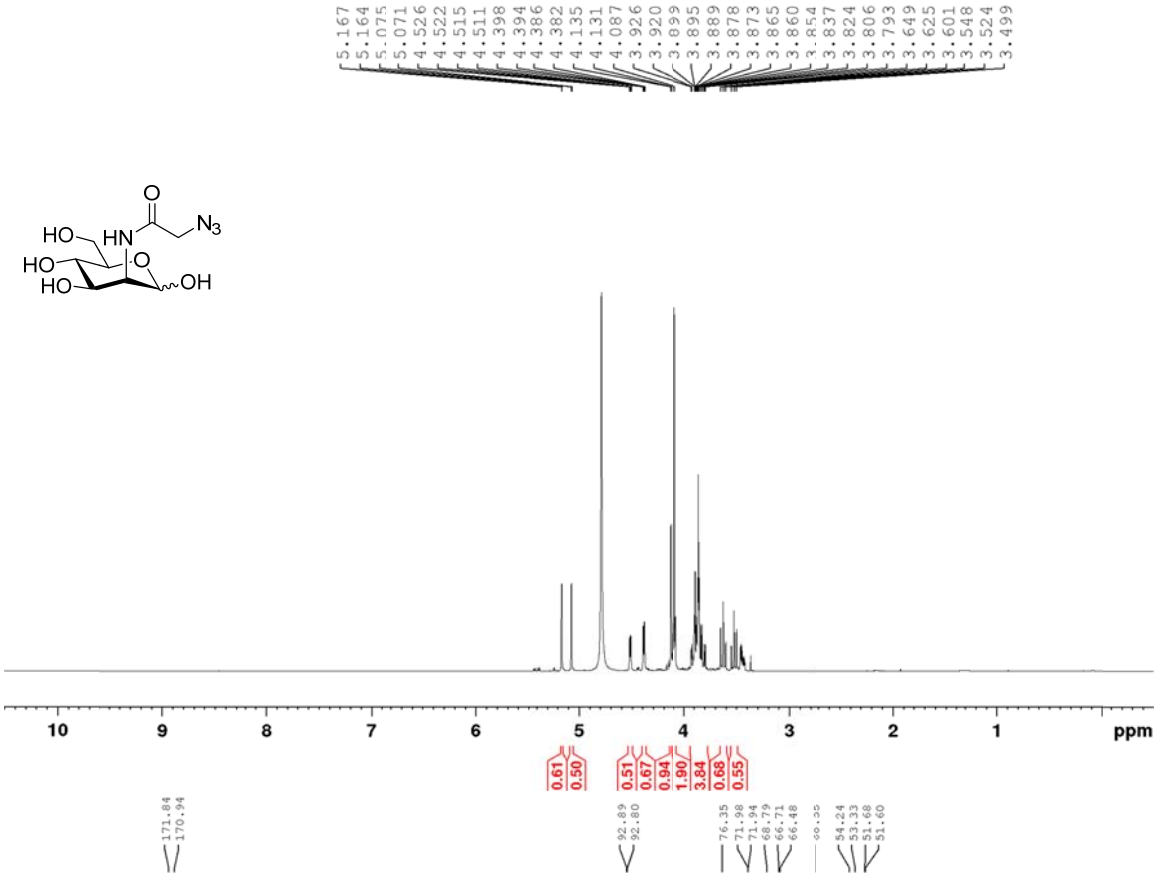
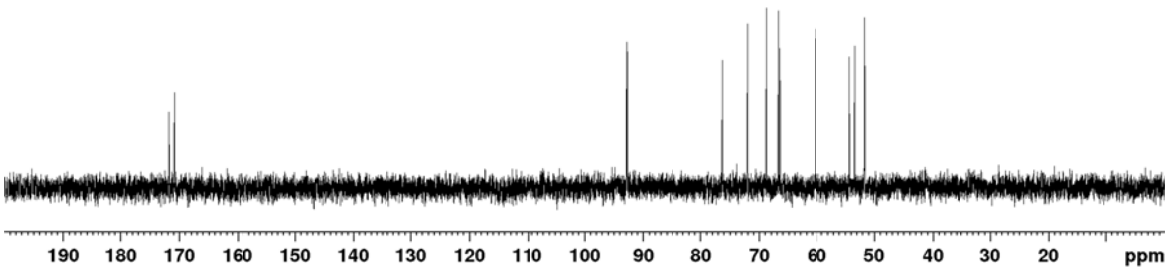


Figure S11. Graph shows fluorescence intensity of SOSG in cells shown in **Figure 5 and Figure S10** (mean ± s.d., n = 3).

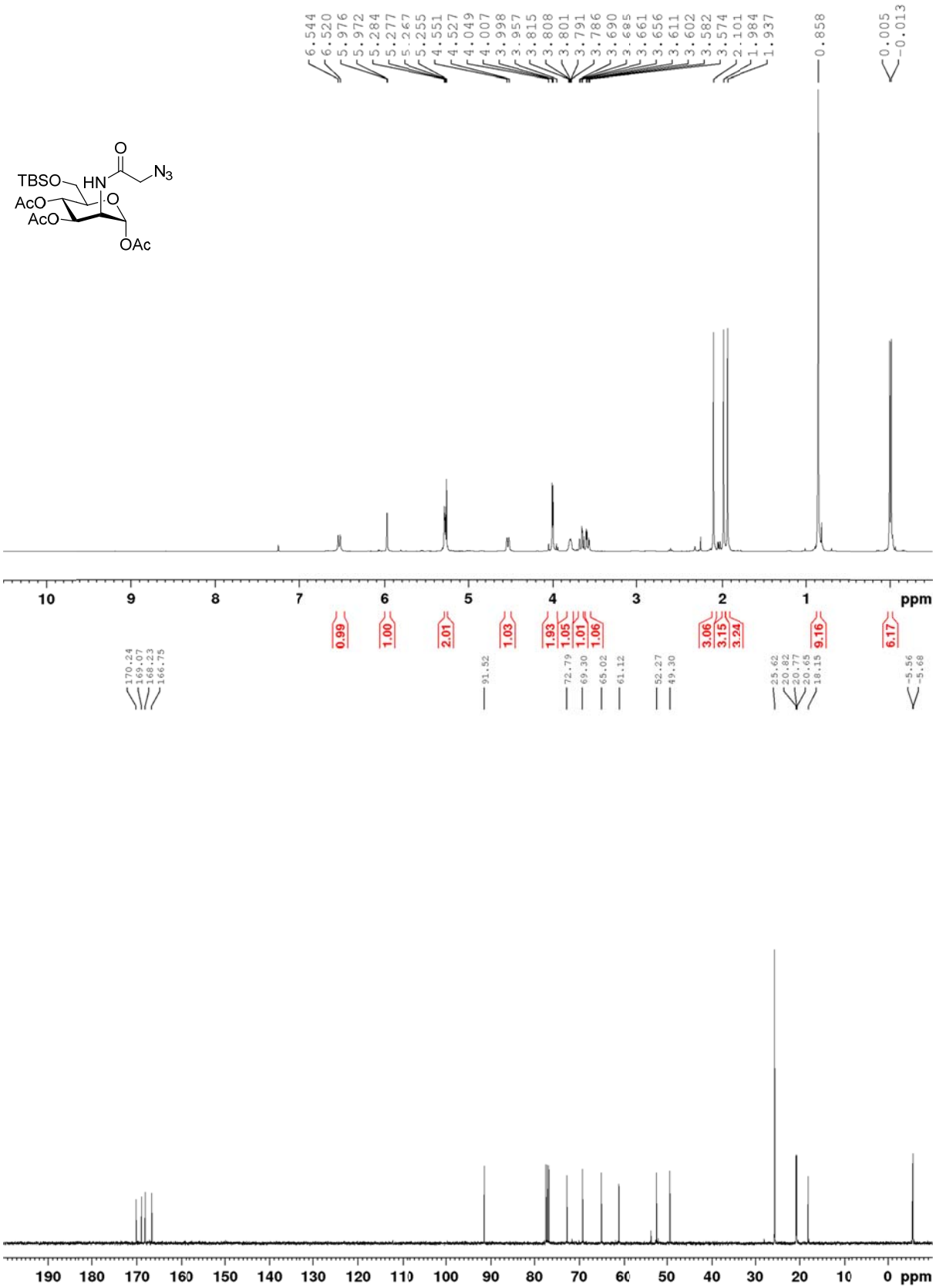
<NMR spectra>

Compound 1 (known, D_2O, 400 MHz ^1H NMR, 100 MHz ^{13}C NMR).

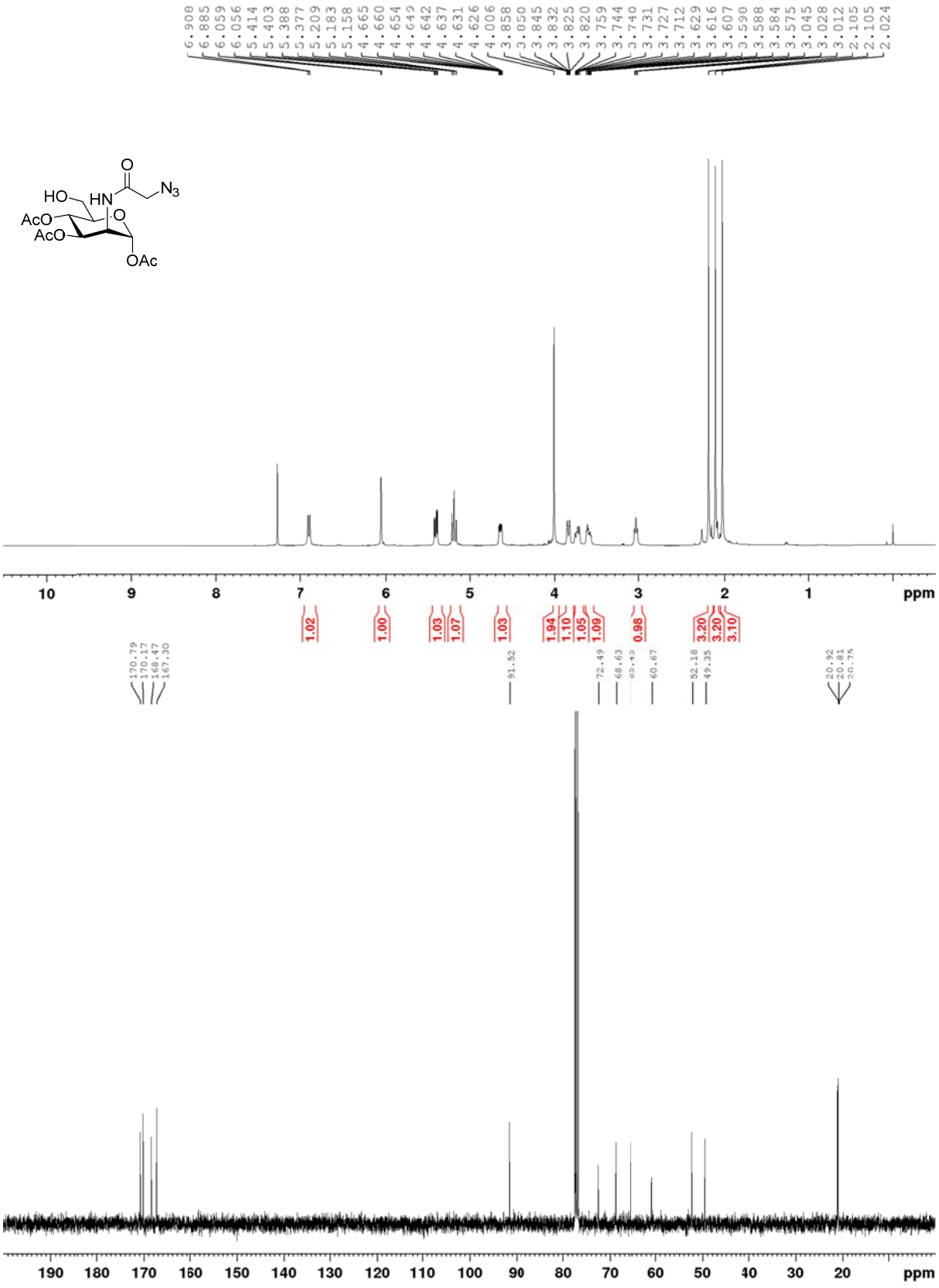




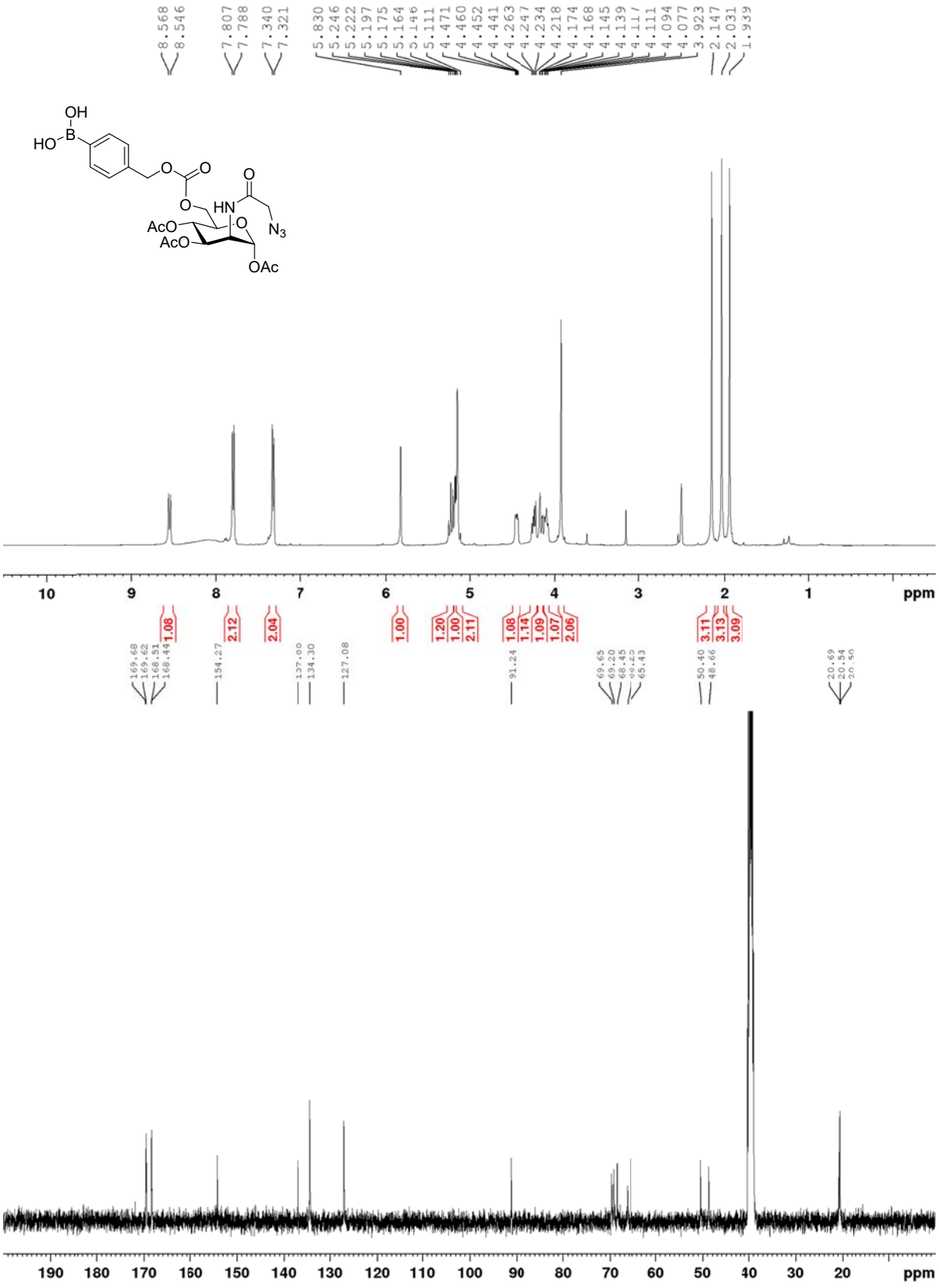
Compound 2 ($CDCl_3$, 400 MHz ^1H NMR, 100 MHz ^{13}C NMR)



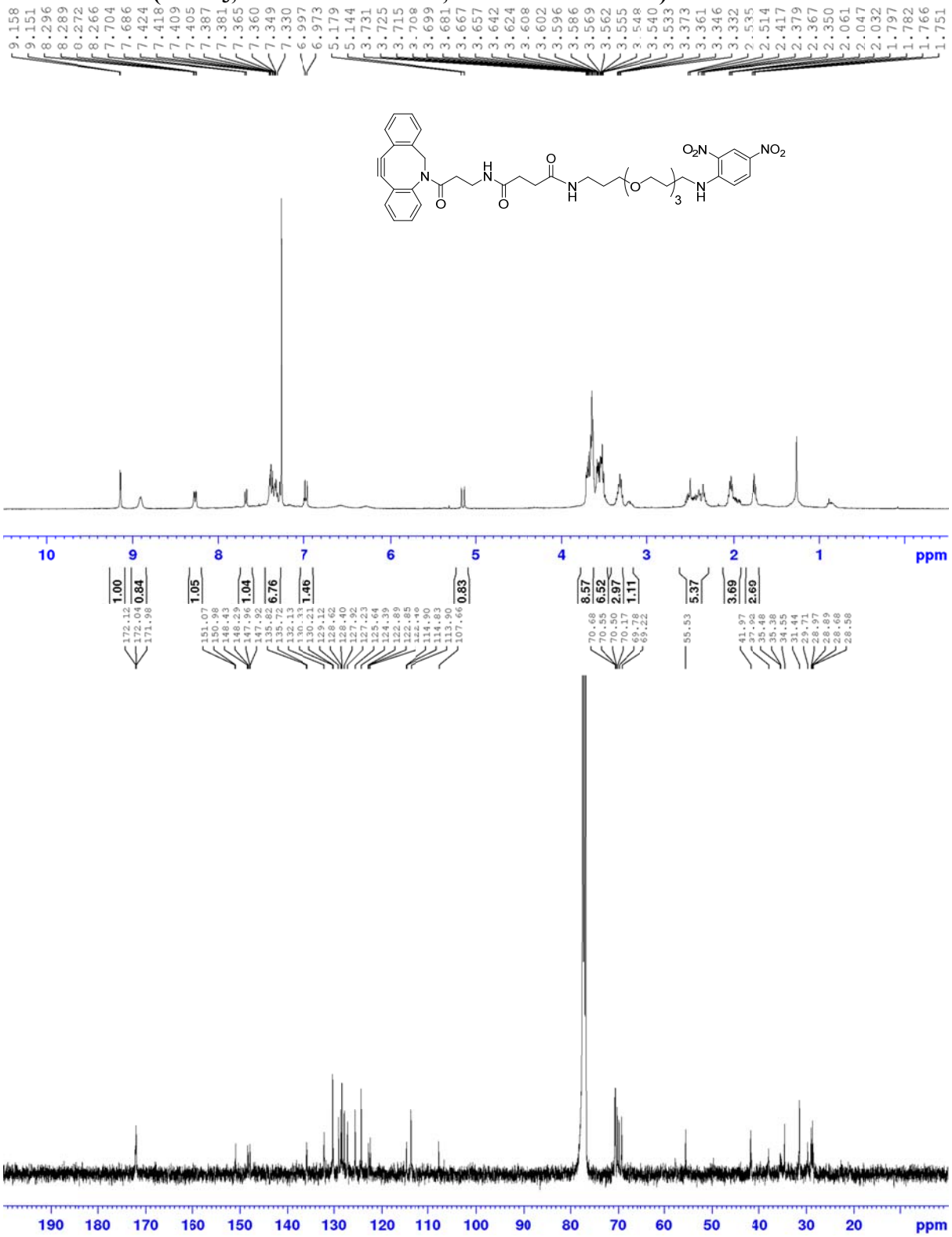
Compound 3 ($CDCl_3$, 400 MHz ^{1}H NMR, 100 MHz ^{13}C NMR)



PBA-ManNAz(OAc)$_3$ (DMSO, 400 MHz ^{1}H NMR, 100 MHz ^{13}C NMR)



ADIBO-DNP ($CDCl_3$, 400 MHz ^1H NMR, 100 MHz ^{13}C NMR)



ADIBO-B (CD_3OD, 400 MHz ^1H NMR, 100 MHz ^{13}C NMR)