Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Identification of Nitric Oxide-Releasing Derivatives of Oleanolic Acid as Potential Anti-Colon Cancer Agents

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Table S1 IC_{50} values (μM) of selected compounds against cancer cell lines[a]

Cell lines	JS-K	PABA/NO	**6**
MCF-7	2.431	5.230	1.696
NIH3T3	1.755	3.451	1.108
HL-60	0.599	2.567	1.571
OVCaR-3	2.927	5.543	3.370
MGC803	5.580	9.311	1.660
HCT-8/V	0.803	13.205	6.258

[a]The inhibitory effects of individual compounds on the proliferation of cancer cell lines were determined by the MTT assay with a 72 h treatment. Data are expressed as mean of three independent experiments.

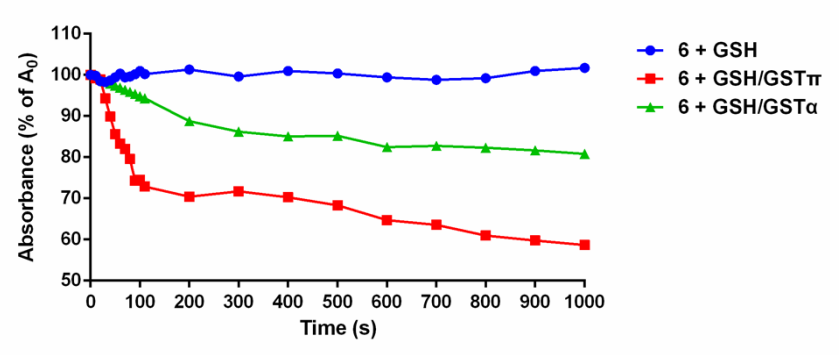

Fig.S1 Representative curves showing the decomposition of **6** (40 μM) in pH 7.4 buffer containing 1 mM GSH with or without a catalytic amount (5 μg/mL) of GST. Decomposition of **6** was measured by monitoring the maximal absorbance of **6** at 339 nm using a microplate reader at room temperature. Data are expressed the percentage of absorbance compared with the initial absorbance (A_0).

General procedure for the preparation of O^2-(2,4-dinitrophenyl)diazeniumdiolates 3b−f. A solution of 1,5-difluoro-2,4-dinitrobenzene (204 mg, 1 mmol) in 15 mL of acetone was cooled to 0 °C under nitrogen. To this was added dropwise a solution of sodium diazeniumdiolate (1.1 mmol) in 15 mL of 5% aqueous sodium bicarbonate. The solution turned bright yellow immediately upon addition, and the resulting mixture was allowed to warm to room temperature and stir until the starting material was totally consumed as indicated by TLC. After the acetone was removed under vacuum, the residue was taken up in CH_2Cl_2 and washed with water. The organic solution was dried over sodium sulfate and evaporated under vacuum to give the desired product, which was recrystallized from ethanol.

***O*²-(2,4-Dinitro-5-fluorophenyl) 1-[(S)-2-hydroxymethyl pyrrolidine-1-yl] diazen-1-ium-1,2-diolate (3b).** The title compound was obtained in 70% yield as a yellow solid: mp 123−124 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.03−2.26 (m, 4H, prolinol), 3.73−3.79 (m, 2H, prolinol), 3.84−3.86 (m, 1H, prolinol), 3.88−3.96 (m, 2H, prolinol), 7.46 (d, J = 11.8 Hz, 1H, ArH), 8.90 (d, J = 7.6 Hz, 1H, ArH); ESI-MS 368.0 $[M + Na]^+$; HRMS calculated for $C_{11}H_{12}FN_5NaO_7$ $[M + Na]^+$ 368.0618, found 368.0620 (parts per million error of 0.4).

***O*²-(2,4-Dinitro-5-fluorophenyl) 1-(4-hydroxypiperidine-1-yl)diazen-1-ium-1,2-diolate (3c).** The title compound was obtained in 61% yield as a yellow solid: mp 128−129 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.78−1.95 (m, 2H, piperidine), 2.00−2.12 (m, 2H, piperidine), 3.61−3.75 (m, 2H, piperidine), 3.83−3.97 (m, 2H, piperidine), 4.00−4.10 (m, 1H, piperidine), 7.44 (d, J = 11.7 Hz, 1H, ArH), 8.93 (d, J = 7.5 Hz, 1H, ArH); ESI-MS 346.3 $[M + H]^+$, 368.0 $[M + Na]^+$; HRMS calculated for $C_{11}H_{13}FN_5O_7$ $[M + H]^+$ 346.0799, found 346.0780 (parts per million error of 0.2).

***O*²-(2,4-Dinitro-5-fluorophenyl) 1-(4-hydroxyethylpiperazin-1-yl)diazen-1-ium-1,2-diolate (3d).** The title compound was obtained in 55% yield as a yellow solid：mp 132−135 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.64 (t, J = 5.2 Hz, 2H, NCH_2), 2.77−2.79 (m, 4H, piperazin), 3.66−3.73 (m, 6H, piperazin, CH_2OH), 7.42 (d, J = 11.6 Hz, 1H, ArH), 8.90 (d, J = 7.5 Hz, 1H, ArH); ESI-MS 375.1 $[M + H]^+$, 387.2 $[M + Na]^+$; HRMS calculated for $C_{12}H_{16}FN_6O_7$ $[M + H]^+$ 375.1065, found 375.1067 (parts per million error of 0.4).

***O*²-(2,4-Dinitro-5-fluorophenyl) 1-(pyrrolidine-1-yl)diazen-1-ium-1,2-diolate (3e).** The title compound was obtained in 62% yield as a yellow solid：mp 115−119 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.05−2.12 (m, 4H, pyrrolidine), 3.78−3.83 (m, 4H, pyrrolidine), 7.43 (d, J = 11.7 Hz, 1H,

ArH), 8.93 (d, J = 7.6 Hz, 1H, ArH); ESI-MS 338.1 $[M + Na]^+$; HRMS calculated for $C_{10}H_{10}FN_5NaO_6$ $[M + Na]^+$ 338.0513, found 338.0516 (parts per million error of 0.6).

***O*²-(2,4-Dinitro-5-fluorophenyl) 1-(piperidine-1-yl)diazen-1-ium-1,2-diolate (3f).** The title compound was obtained in 68% yield as a yellow solid: mp 122−125 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.79−1.86 (m, 6H, piperidine), 3.65 (t, J = 5.6 Hz, 4H, piperidine), 7.50 (d, J = 14.5 Hz, 1H, ArH), 8.91 (d, J = 7.6 Hz, 1H, ArH); ESI-MS 347.1 $[M + NH_4]^+$, 352.1 $[M + Na]^+$; HRMS calculated for $C_{11}H_{13}FN_5O_6$ $[M + H]^+$ 330.0850, found 330.0852 (parts per million error of 0.4).

General procedure for the preparation of compounds 15a and 15b. A mixture of compound **14** (786 mg, 1 mmol), *N*-Cbz-L-proline or *N*-Cbz-*N*-methylglycine (1.5 mmol), EDCI (288 mg, 1.5 mmol), and DMAP (122 mg, 1 mmol) in anhydrous CH_2Cl_2 (15 mL) were stirred at room temperature for 12 h. An additional 100 ml of CH_2Cl_2 was then added, and the mixture was washed sequentially with 2 N HCl (3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL), and saturated NaCl solution. The organic fraction was dried over sodium sulfate. After removal of the solvent, the crude products were purified by column chromatography [1:3 (v/v) ethyl acetate/petroleum] to give the title compounds.

Compound 15a. The title compound was obtained in 88% yield as a white solid: mp 116−120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.91 (s, 6H, 2 × CH_3), 0.94 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.99 (s, 3H, $COCH_3$), 2.03 (s, 6H, 2 × $COCH_3$), 2.16 (s, 3H, $COCH_3$), 2.23−2.45 (m, 4H, proline), 2.80−2.84 (m, C_{18}-H), 3.74−3.80 (m, 3H, proline), 3.97 (d, J = 5.0 Hz, 1H, H_3), 4.10−4.12 (m, 2H, H_4, H_5), 4.57 (t, J = 7.9 Hz, 1H, 3α-H), 5.06−5.10 (m, 1H, H_2), 5.12 (s, 2H, Ph*CH₂*), 5.30 (brs, 1H, C_{12}-H), 5.33−5.35 (m, 1H, $H_{6'}$), 5.38−5.41 (m, 1H, H_6), 5.55 (d, J = 8.2 Hz, 1H, H_1), 7.30−7.35 (m, 5H, ArH); ESI-MS 1018.5 $[M + H]^+$, HRMS calcd for $C_{57}H_{80}NO_{15}$ $[M + H]^+$ 1018.5450, found 1018.5452 (parts per million error of 0.2).

Compound 15b. The title compound was obtained in 92% yield as a white solid: mp 110−113 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75 (s, 3H, CH_3), 0.84 (s, 6H, 2 × CH_3), 0.90 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 2.00 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 2.80−2.84 (m, C_{18}-H), 3.32 (s, 3H, NCH_3), 3.99−4.03 (m, 1H, H_3), 4.05 (s, 2H, NCH_2), 4.10−4.13 (m, 2H, H_4, H_5), 4.48−4.59 (m, 1H, 3α-H), 5.06−5.10 (m, 1H, H_2), 5.11 (s, 2H, Ph*CH₂*), 5.32 (brs, 1H, C_{12}-H), 5.35−5.39 (m, 1H, $H_{6'}$), 5.40−5.42 (m, 1H, H_6), 5.55 (d, J = 8.3 Hz, 1H, H_1), 7.31−7.35 (m, 5H, ArH); ESI-MS

992.6 $[M + H]^+$; HRMS calcd for $C_{55}H_{78}NO_{15}$ $[M + H]^+$ 992.5293, found 990.5297 (parts per million error of 0.4).

General procedure for the preparation of compounds 16a and 16b. The corresponding compounds **15a** and **15b** were dissolved in a 1:1 mixture of anhydrous CH_2Cl_2 and MeOH at ice-bath temperature, and the pH was adjusted to 9.0 using 0.1 N MeONa/MeOH. The deacetylation procedure was monitored by TLC, and upon completion the pH was adjusted to 7.0 with acidic ion exchange resin 001 × 7 (732). After filtration, the filtrate was evaporated in vacuo, and the resulting residue was purified by column chromatography [1:10 (v/v) MeOH/CH_2Cl_2] to furnish the respective title product.

Compound 16a. The title compound was obtained in 90% yield as a white solid: mp 123−127 °C; ^{1}H NMR (d_6-DMSO, 300 MHz) δ 0.79 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.88 (s, 9H, 3 × CH_3), 1.10 (s, 3H, CH_3), 2.20−2.42 (m, 4H, proline), 2.74−2.77 (m, C_{18}-H), 3.36−3.54 (m, 5H), 3.65−3.80 (m, 5H), 4.42−4.44 (m ,1H, OH), 4.49 (d, J = 4.3 Hz, 1H, OH), 4.54−4.56 (m, 1H, 3α-H), 4.75 (d, J = 5.3 Hz, 1H, OH), 4.97 (d, J = 5.6 Hz, 1H, OH), 5.04 (s, 2H, PhCH_2), 5.17 (brs, 1H, C_{12}-H), 5.22 (d, J = 7.8 Hz, 1H), 7.35−7.64 (m, 5H, ArH); ESI-MS 850.5 $[M + H]^+$, 872.5 $[M + Na]^+$; HRMS calcd for $C_{49}H_{72}NO_{11}$ $[M + H]^+$ 850.5027, found 850.5031 (parts per million error of 0.4).

Compound 16b. The title compound was obtained in 87% yield as a white solid: mp 122−125 °C; ^{1}H NMR (d_6-DMSO, 300 MHz) ^{1}H NMR (d_6-DMSO, 300 MHz) δ 0.69 (s, 3H, CH_3), 0.79 (s, 6H, 2 × CH_3), 0.88 (s, 9H, 3 × CH_3), 1.10 (s, 3H, CH_3), 2.73−2.77 (m, C_{18}-H), 3.32 (s, 3H, NCH_3), 3.37−3.51 (m, 5H), 3.68 (s, 1H), 3.98−4.12 (m, 1H, 3α-H), 4.02 (s, 2H, NCH_2), 5.01 (s, 2H, PhCH_2), 5.17 (brs, 1H, C_{12}-H), 5.21 (d, J = 7.6 Hz, 1H), 7.21−7.43 (m, 5H, ArH), 7.73 (d, J = 6.4 Hz, 1H, NH); ESI-MS 824.3 $[M + H]^+$, 846.5 $[M + Na]^+$; HRMS calcd for $C_{47}H_{70}NO_{11}$ $[M + H]^+$ 824.4871, found 824.4875 (parts per million error of 0.4).

HPLC assessment of compound purity.

All tested compounds (**5**−**13**) with a purity of > 95% were used for subsequent biological assays. We provided the spectra of HPLC assays as below.

Column: Inertex C18 (150 mm × 4.6 mm × 5 μm);

Mobile phase: Methanol: Water = 80:20 (v/v);

Rate: 1 mL/min;

Temperature: 25 °C;

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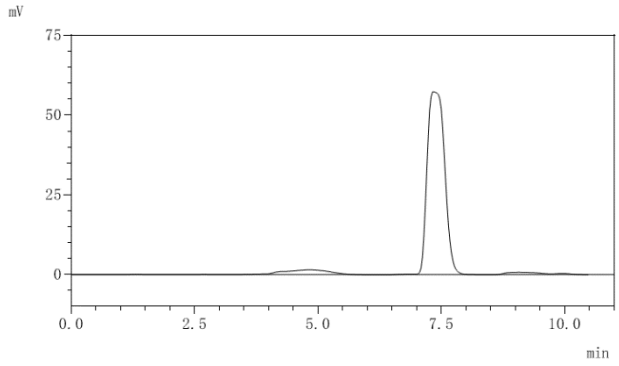

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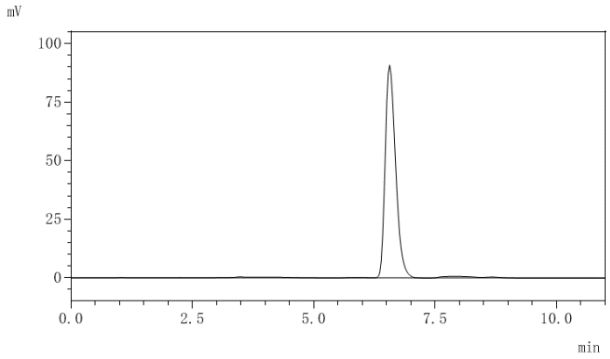

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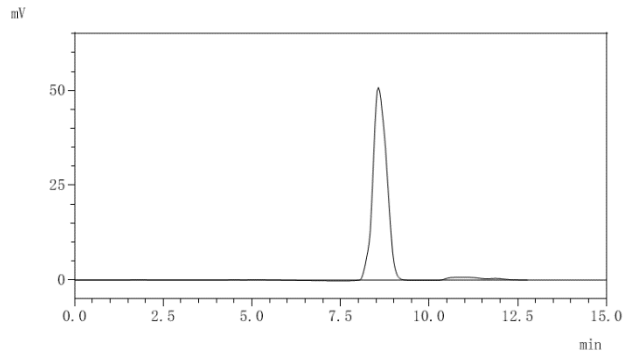

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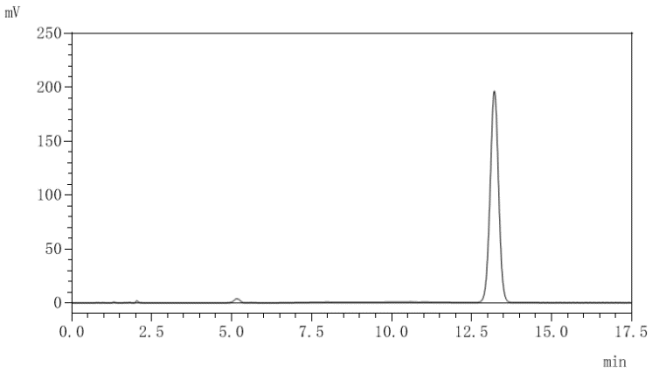

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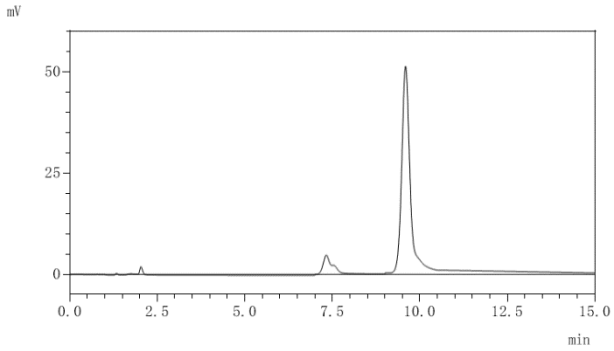

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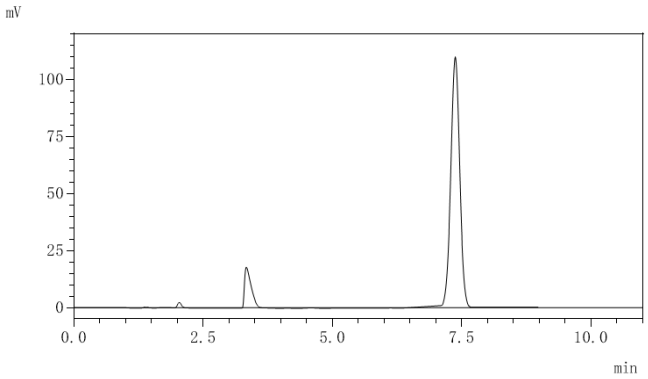

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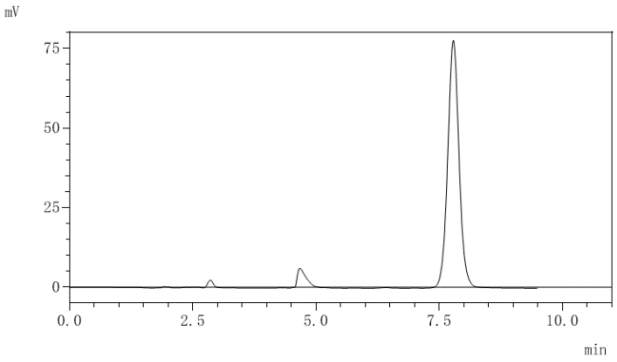

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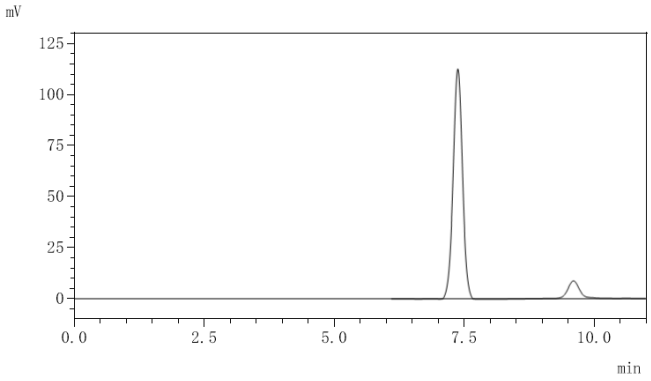

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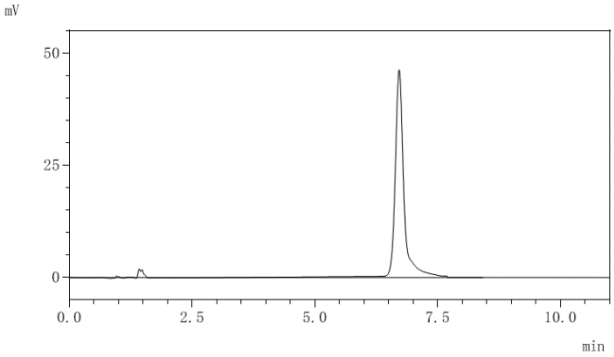

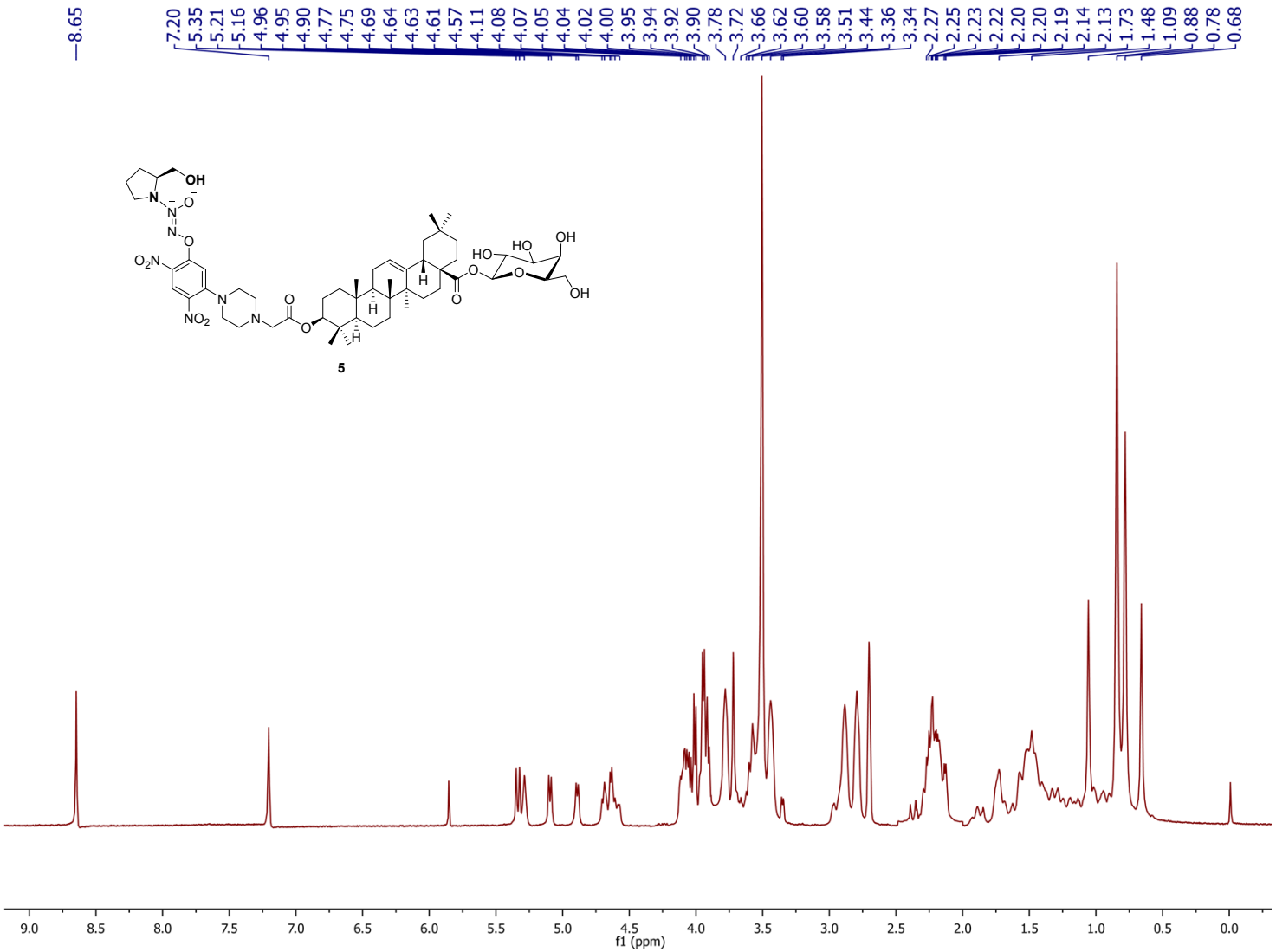
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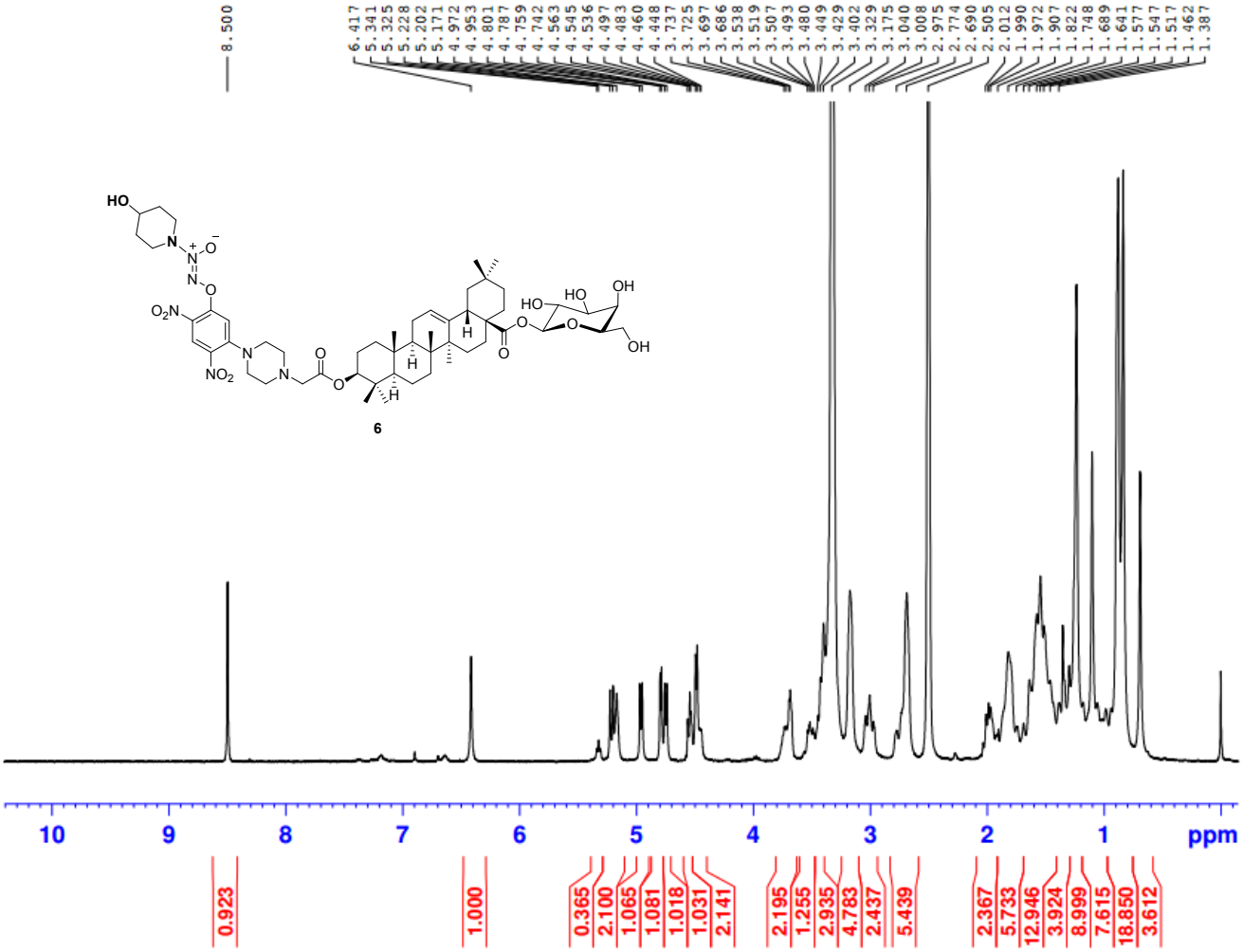
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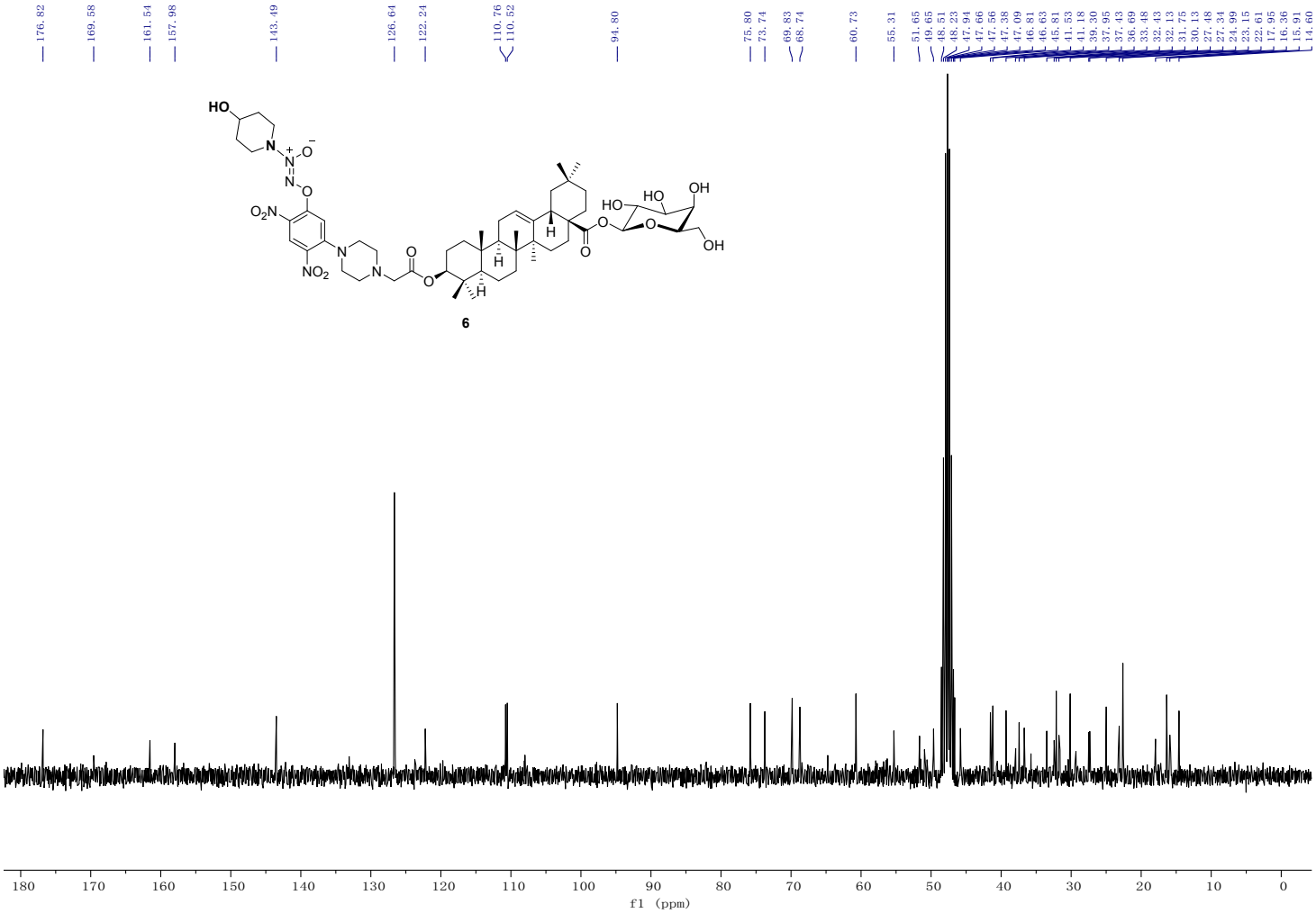
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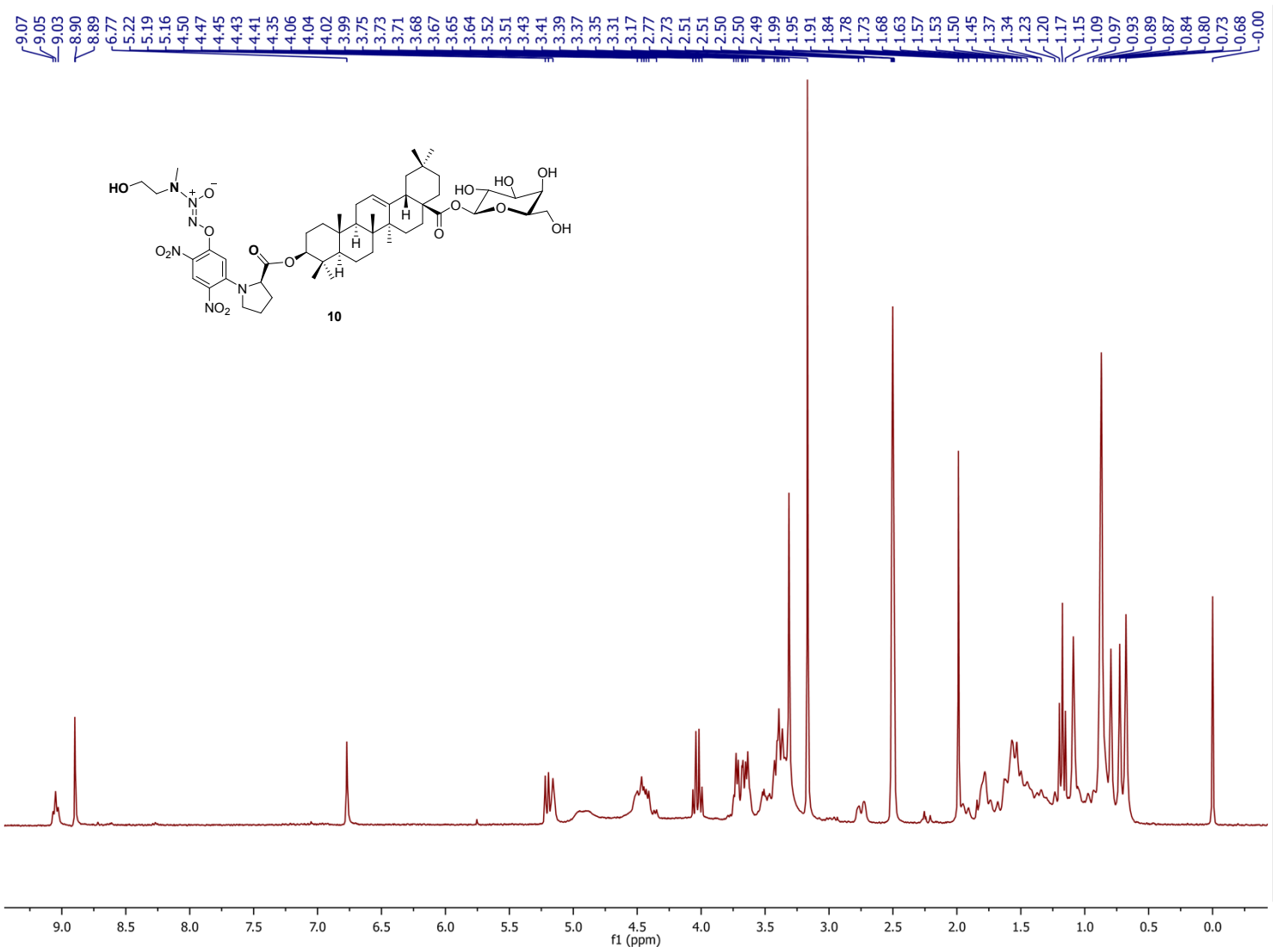
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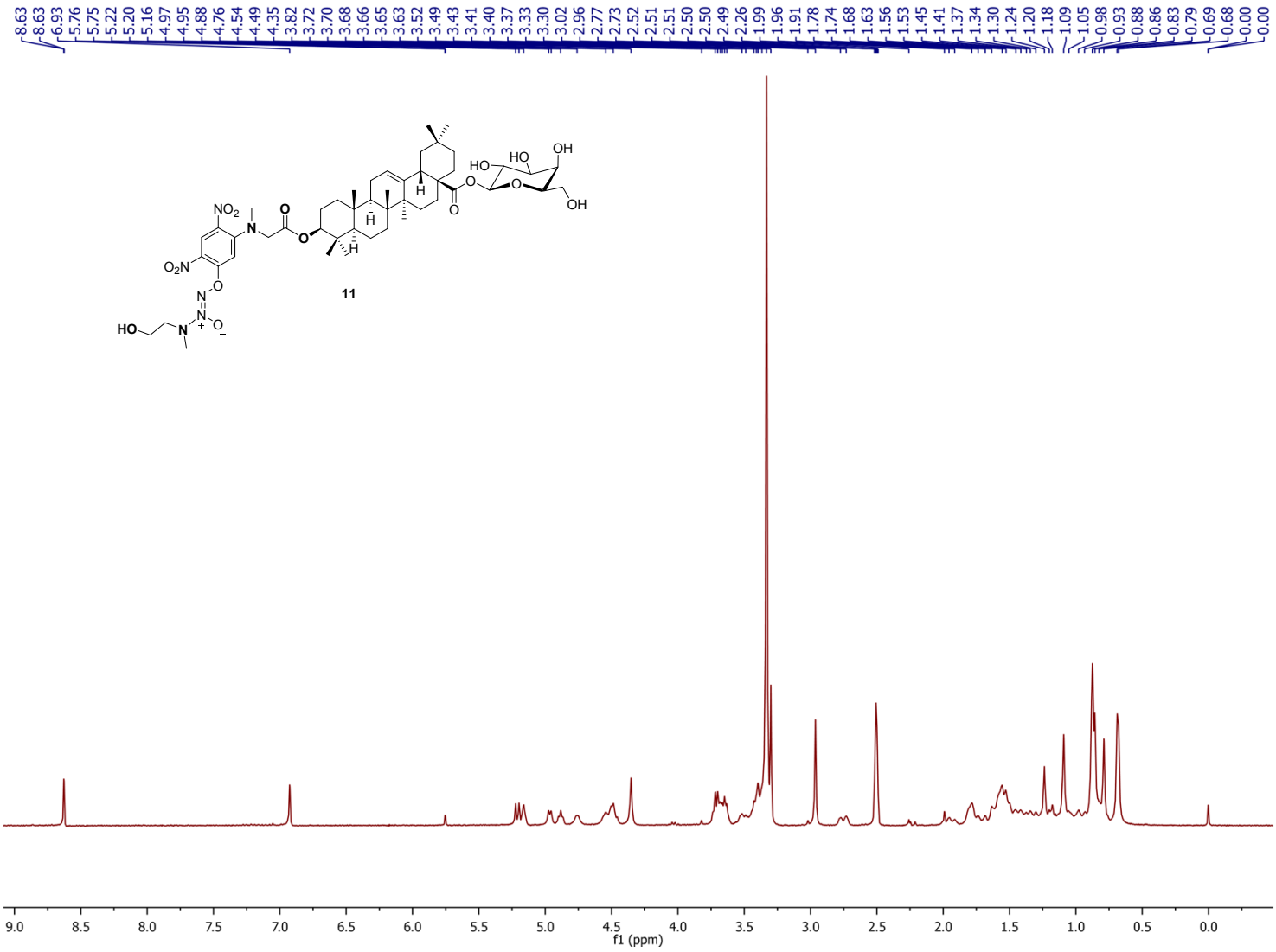
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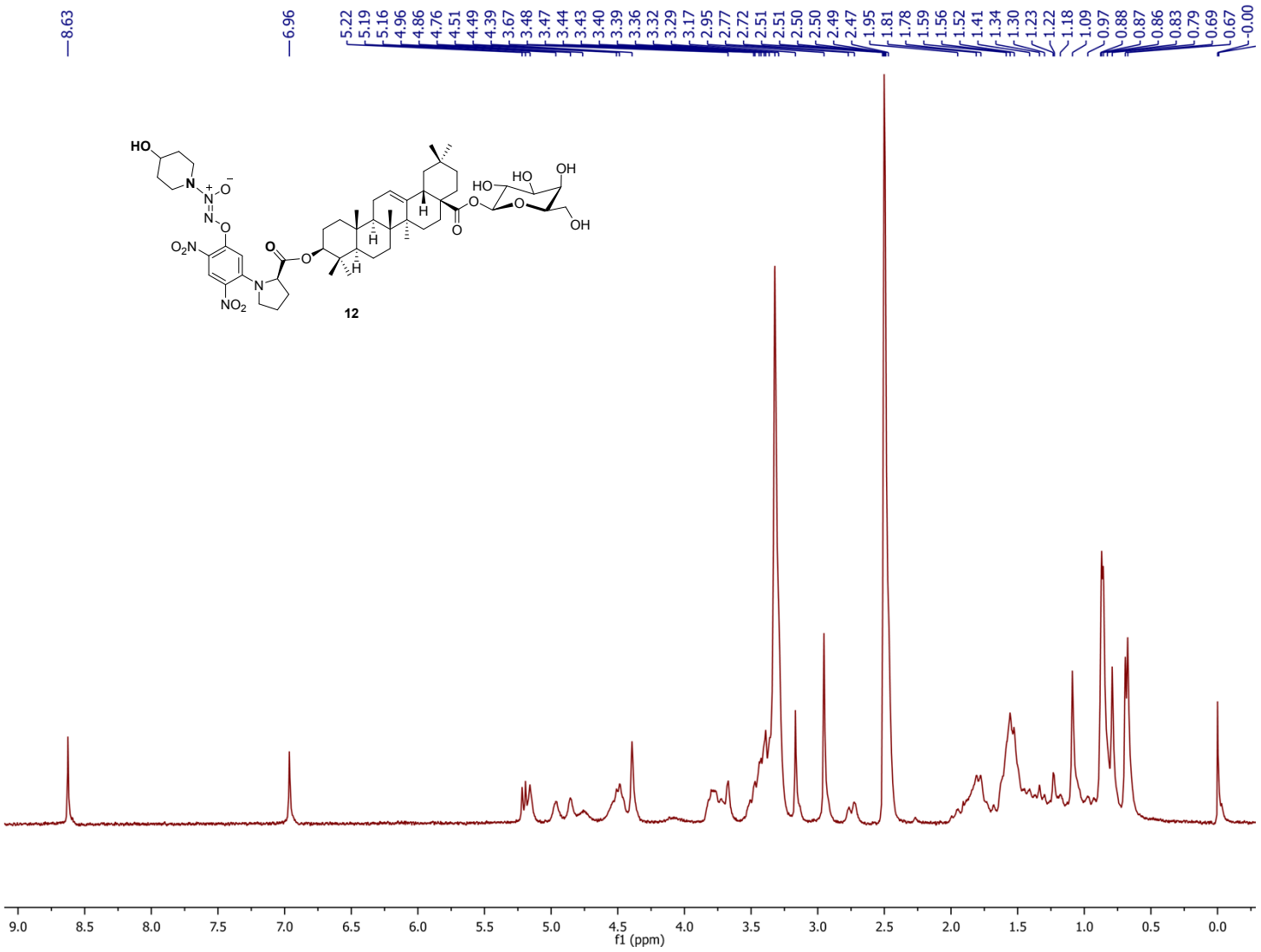
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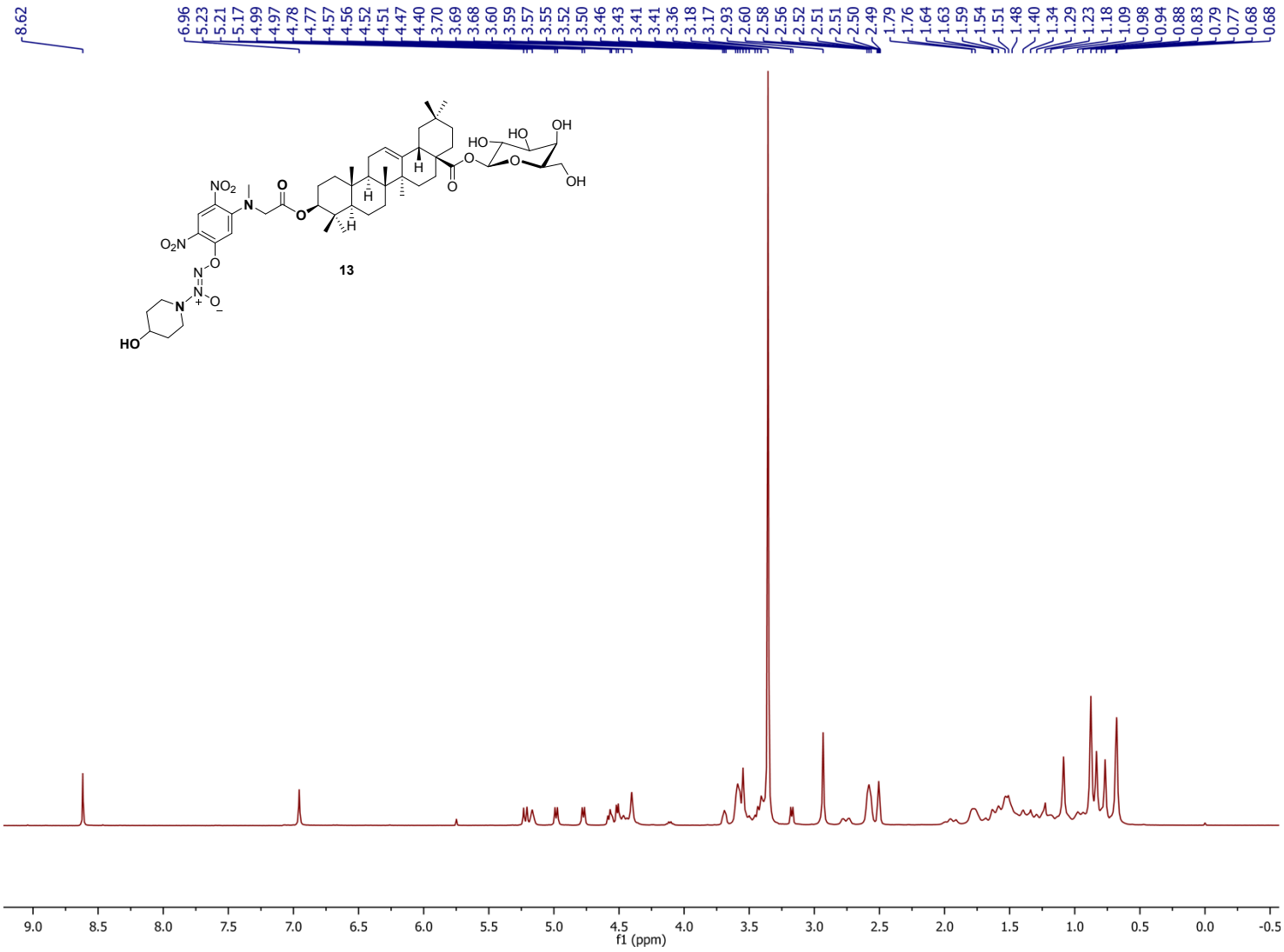
1.78
1.59
1.56
1.52
1.41
1.34
1.30
1.23
1.22
1.18
1.09
0.97
0.88
0.87
0.86
0.83
0.79
0.69
0.67
-0.00
HO
N
O
N
O
O2N
NO2
O
O
N
H
H
HO
HO
OH
O
O
OH
O
12
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


8.62
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5.23
5.21
5.17
4.99
4.97
4.78
4.77
4.57
4.56
4.52
4.51
4.47
4.40
3.70
3.69
3.68
3.60
3.59
3.57
3.55
3.52
3.50
3.46
3.43
3.41
3.41
3.36
3.18
3.17
2.93
2.60
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2.56
2.52
2.51
2.51
2.50
2.49
1.79
1.76
1.64
1.63
1.59
1.54
1.51
1.48
1.40
1.34
1.29
1.23
1.18
1.09
0.98
0.94
0.88
0.83
0.79
0.77
0.68
0.68
NO2
O2N
HO
HO
HO
OH
OH
13
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)